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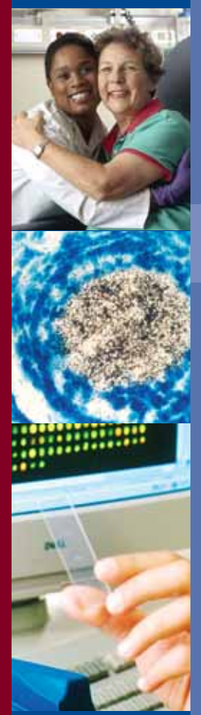
The Early Detection Research Network



Division of Cancer Prevention

The Early Detection Research Network

Investing in Translational Research on Biomarkers of Early Cancer and Cancer Risk

FOURTH REPORT • JANUARY 2008

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

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"As chairperson and founder of a cancer non-profit focused on barriers and opportunities to furthering cancer research, I see the impact of the Early Detection Research Network first-hand. The collaborative nature of EDRN is essential to our community's success; it is critical we work together — across disciplines, across industries and across the aisle — to find ways to detect and treat cancer earlier. Recently, our organization co-hosted a town hall with the University of Michigan Comprehensive Cancer Center in Ann Arbor that featured an expert panel of UM's leading cancer researchers; Congressman John Dingell (D-MI); Dr. John Niederhuber, Director of the National Cancer Institute (NCI); and Dr. Andrew von Eschenbach, former NCI Director and current Commissioner of the Food and Drug Administration. One of the most exciting discussions centered on the promising research being conducted on biomarkers as a tool for individualized detection, prevention and treatment of cancer. From having the privilege of serving on the National Cancer Institute's Board of Scientific Advisors, I have seen the pivotal role of EDRN in bringing biomarkers to clinical application. The ultimate impact on public health will be invaluable."

Ellen V. Sigal, Ph.D.
Chairperson, Friends of Cancer Research
Arlington, Virginia

National Cancer Institute

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Foreword

January 2008

In 2000, NCI's Division of Cancer Prevention created an investigator-driven network designed to conduct translational research that identified markers both for the early detection of cancer and of cancer risk. That program, the Early Detection Research Network (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 divergent disciplines, including genomics, proteomics, metabolomics, bioinformatics and public health.

EDRN is at the forefront of technology-driven research on the 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, progression of disease, or response to therapy. Ultimately, EDRN research will aid in prevention and in early therapeutic intervention, based on early detection of disease.

Researchers with EDRN have been instrumental in identifying and validating markers for many major cancers, such as prostate (protein profiling of BPH, HPIN and IGFb3/br), colon (K-ras mutations in stool and urine) and breast (alpha catenin genes). They have also joined forces with clinical trial communities to accelerate biomarker validation. To take just one example, EDRN investigators work with investigators in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial and in the Specialized Programs of Research Excellence (SPORE) program, to test a panel of biomarkers for ovarian cancer in sera collected in the PLCO trial.

Early detection can dramatically improve outcomes. Finding breast and colon cancers when they remain localized results in 5-year survival rates of 90 percent or higher. EDRN is helping make that an achievable goal for more and more cancers.

John Niederhuber, M.D.
Director
National Cancer Institute
National Institutes of Health

Introduction

NCI's Division of Cancer Prevention set out 7 years ago to create a strong, investigator-driven network to conduct translational research to identify tests for early cancer and cancer risk. In 2000, the Early Detection Research Network (EDRN) became a fully funded group of 28 grantees focused on the overarching goal of creating validated biomarkers ready for large-scale clinical testing.

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 benefit.

These 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, as 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 with a variety of cancers, acceptable biomarkers must be: reliable and repeatable 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 the chemopreventive agent; and have high predictive value for clinical disease.

EDRN is helping translate the discovery and validation of biomarkers to clinical use and we are delighted to be working toward that end.

Peter Greenwald, M.D., Dr.P.H., Director
Division of Cancer Prevention, National Cancer Institute
Assistant Surgeon General, U.S. Public Health Service

Executive Summary

The National Cancer Institute (NCI) is bringing visionary people together through research collaborations that inspire innovative approaches to early detection, prevention and treatment of cancer.

NCI launched the Early Detection Research Network (EDRN) (http://edrn.nci.gov/) in 2000 to identify biomarkers, substances found in blood, body fluids or tissue that show the risk or presence of disease before cancer has had the opportunity to progress in the body. EDRN is the only program focused directly on the discovery and validation of biomarkers for noninvasive, early detection of cancer. The Network unites clinical and basic scientists so that discovery is clinically driven, yet balanced with a systematic approach to validation.

Table 1. Early Detection Tests for Cancer, Selected Organ Sites

Organ Site	Test
Bladder	None
Breast	Mammogram
Cervix	Pap smear
Colorectal	Fecal occult blood test, sigmoidoscopy, colonoscopy, double contrast barium enema, digital rectal exam
Esophageal	None
Kidney	None
Liver (primary)	None, but two molecular tests are approved for risk assessment
Lung	Imaging
Ovary	None proven to decrease mortality
Pancreatic	None
Prostate	None proven to decrease mortality

Recent reductions in cancer mortality are due in part to risk reduction behaviors like smoking cessation and more strongly to early detection of cancer coupled with appropriate therapy. Yet, there are no validated molecular biomarker tests for the early detection of any cancer (see Table I). Among the list of Food and Drug Administration (FDA)-approved biomarkers, none have been approved for cancer early detection and screening. EDRN is studying more than 120 biomarkers for the major organ system groups (see Table 2), some of which are in Phase 3 testing, a retrospective longitudinal approach that determines how well biomarkers detect preclinical disease by testing them against tissues collected longitudinally from research cohorts.

Investigators from more than 40 research institutions are part of the Network. All share a common belief that the integration of discovery, evaluation and clinical validation phases of medical research are more likely to succeed when they are carried out in a concerted and systematic fashion. A common problem is that after researchers discover biomarkers, the biomarkers are not produced for clinical use because they have not been validated in other laboratories. To address this, EDRN drew up and implemented standards to accelerate the progress for discovering and validating reproducible biomarkers that ultimately can be moved on to clinical use.

Through cooperative agreement awards, NCI is closely involved in the EDRN projects to ensure the studies gather necessary data. EDRN welcomes other interested researchers to join the Network through smaller scale

projects. The Network is challenged to motivate scientists to offer their candidate biomarkers for testing and to educate scientists about the importance of rigorous prevalidation studies that prepare the way for successful biomarker validation.

This report, the fourth in a series, summarizes the major developments in the Network since its inception through a discussion of concepts and concrete examples, beginning with a historical and structural overview. It also shows how progress has occurred in the areas of:

- Disease-specific advancements across the major organ sites;
- Process and collaboration; and
- An adaptive business model approach that encourages public-private partnerships and team science.

Table 2. Early Detection Biomarkers in Study for Selected Cancer Sites 2003 to 2007 (partial list; see organ specific chapters for details)

Site	Number of Biomarkers *
Bladder	3
Breast	7
Cervical /Endometrial	2
Colorectal	21
Esophagus	7
Hepatocellular	9
Kidney	1
Lung	12
Mesothelium	2
Ovarian	5
Pancreatic	16
Prostate	15

* Panels including more than one biomarker were counted as one.

Disease-Specific Advancements

EDRN has active ongoing work in cancer sites that constitute nearly 1 million cancer diagnoses each year and more than 350,000 deaths.

Biomarkers in development by EDRN address common malignancies as well as mesothelioma and hepatocellular cancer. The latter are of major worldwide importance and are increasing in incidence in the United States. EDRN Collaborative Groups, focused on breast and gynecologic cancers, gastrointestinal and other associated cancers, lung and upper aerodigestive cancers and prostate and urologic cancers, each have biomarkers in prevalidation and validation phases in which the accuracy of experimental results is confirmed.

There are over 120 biomarkers in development, alone and in combinations, across the EDRN phases: 27 in Phase 2 development (validating the capacity of biomarkers to distinguish between people with cancer and those without), of which, more than 15 are progressing toward Phase 3; and five in Phase 3 development (determining the capacity to detect preclinical disease).

Highlights of EDRN achievements include:

- Standard reference specimens and reagents, primarily plasma and serum (cases and matched controls) were developed for detection and evaluation of prostate cancer biomarkers; urine reference sets are being developed for bladder, prostate, colon and lung cancers.
- Recurrent non-random chromosomal translocations were discovered in prostate cancer along with some other potential markers, such as %proPSA, PCA3, AMACR and a panel of autoantibodies; panels of methylated DNA sequences and other biomarkers have been identified as promising biomarkers for bladder and prostate cancers; and mutations and deletions in mitochondrial DNA were detected in prostate and other cancers.
- Molecular tests for ovarian cancer are progressing towards validation; one of the tests included a panel of markers consisting of MIF-1, prolactin, osteopontin, IGF-2, leptin, HE-4 and others. Studies are underway targeting pre-cancers of the cervix to improve outcomes and reduce treatments; and novel strategies against breast cancer, including early detection using blood markers, will be tested in the next year.

- For each digestive cancer organ site (colon, rectum, esophagus, liver and pancreas), new biomarkers have been discovered and, in prevalidation studies, have been shown to be superior to current standards of care. Two of these biomarkers for colorectal cancer, CCSA-2 and CCSA-3 and two biomarkers for liver cancer, DCP and AFP-L3, are now in clinical validation.
- Work is advancing to identify and validate non-invasive biomarkers in blood or sputum for the early detection of lung cancer, which could be combined with CT scanning of the lung or other imaging methods. In two preliminary blinded experiments, a panel of only two marker genes readily identified lung cancers at specificity and sensitivity values exceeding those of conventional cytology by two to three times.
- Investigators supported through various funding mechanisms (e.g., EDRN, R01, P01 and Specialized Programs of Research Excellence (SPOREs)) have formed a Lung Cancer Biomarkers Working Group. This group is developing and validating proteomics-based biomarkers for early detection of lung cancer and collaborating with other researchers by providing statistically powered specimen sets for rapid evaluation of emerging technologies and biomarkers.

Some biomarker discoveries are performed in tandem with prevalidation studies using high-quality specimens made available to investigators by other NIH supported programs, such as the Women's Health Initiative (WHI) for a colon cancer project; the Carotene and Retinol Efficacy Trial (CARET) for a lung cancer and mesothelioma project; and the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO) for an ovarian cancer project. Leads on other biomarkers from model systems are being tested in humans.

Process and Collaboration

Validation of biomarkers is a formidable task, which needs a consistent approach. EDRN-supported validation studies are, therefore, remarkable achievements. Few biomarkers and developmental laboratories ever achieve the requirements necessary to conduct such studies. But EDRN brings to the table both the scientific paradigm and the ability to effectively organize the resources. Five case-control studies described in this report illustrate this capacity. EDRN also adopted criteria to prioritize analytical and clinical validation studies.

Quality assurance is integral to EDRN. The Network established five Biomarker Reference Laboratories (BRLs) to support clinical and analytical validation efforts: the University of California, Los Angeles (UCLA), University of Alabama, Birmingham (UAB), Johns Hopkins University (JHU), the University of Maryland (UM) and the National Institute of Standards and Technology (NIST). The BRLs are important resources for technology development, standardization of biomarkers and the refinement of existing methods. Some BRL projects include:

- Validation of bleomycin-induced chromosomal breakage in lymphocytes as markers of lung cancer susceptibility;
- Validation of mitochondrial DNA mutations as an early detection marker;
- Development of high-density breast and prostate tissue microarrays;
- Validation of saliva-based assay for oral cancer, refinement of ELISA-based assay for ovarian biomarker panel;
- Validation of standard operating procedures, MSA assays, methylation assays; and
- Validation of several prostate-specific biomarkers, assays and proteomics-based discoveries.

EDRN develops and optimizes technologies for biomarker research. Innovative methods to identify gene alterations, gains and mutations and mitochondrial DNA mutations have been used. Proteomics, auto-antibodies, microsatellite analyses, immunohistochemical markers, polymerase chain amplification of RNA and glycobiology are also employed.

Advances were made in deploying and expanding an informatics framework to support information management. Accessing the information includes specific annotations of markers, the capture of scientific data, management of the study-specific information

and a scientific portal. A major new release integrated with a scientific portal was deployed in 2007.

One of the signature accomplishments of the informatics team is the development of common data elements (CDEs) for use among the EDRN Clinical Epidemiology and Validation Centers (CEVCs). CDEs capture and share data among centers. State-of-the-art methods that previously did not exist have been established for data elements, e.g. acquisition and storage of biologicals, study design, outcome assessment and biomarker validation.

Each EDRN institution within the knowledge system uses CDEs to describe critical cancer data objects and to map their local data models to the Network's knowledge system, in turn providing Network-wide semantic consistency. At the same time, the EDRN Network Exchange system (ERNE) unified search and retrieval of biospecimen data from all institutions regardless of their location, how it is stored, or the differences in the underlying data models. This enables a scientist, for example, to locate tissue specimens for breast cancer by searching data catalogs at participating EDRN institutions across the country.

EDRN-supported statistical tools and informatics infrastructure make the sharing of samples, the developing of collaborations and the exchanging of information with the extramural community at-large, both feasible and productive. The EDRN informatics efforts were cited as a model in reports by the National Academy of Sciences Institute of Medicine, *Developing Biomarker-Based Tools for Cancer Screening, Diagnosis and Therapy: The State of the Science, Evaluation, Implementation and Economics* (Margie Patlak and Sharyl Nass, 2006) and *Cancer Biomarkers: The Promises and Challenges of Improving Detection and Treatment*, (Sharyl J. Nass and Harold L. Moses, Editors, 2007).

EDRN developed a secure, web-based system, the Validation Studies Information Management System (VSIMS), to manage the necessary components for capturing and preserving the 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.

EDRN is establishing a science data warehouse, which will act as a distributed metadata-driven system to capture, track, process and retrieve scientific data from biomarker validation studies and to share across institutions. The EDRN Knowledge System promises to dramatically improve the capability for scientific research by enabling real-time access to a variety of information across research centers.

Adaptive Business Model

The core of EDRN's achievements is the Vertical Adaptive Business Model. This structure encourages public-private partnerships and team science. EDRN promotes a vertical approach for conducting biomarker research, whereby biomarkers are developed in BDLs, refined and cross validated by Biomarker Reference Laboratories (BRLs) and validated in collaboration with CEVCs, all within one organization. The focus is on coordinating multiple resources with a goal of minimizing the barriers to the rapid and efficient "hand-off" between entities.

Five federal agencies—NIST, the Centers for Disease Control and Prevention, FDA, the Pacific Northwest National Laboratories of the Department of Energy and the National Aeronautics and Space Administration (NASA) Jet Propulsion Laboratory (JPL)—participate with EDRN through interagency agreements. Other intergovernmental collaborative partnerships include the National Heart, Lung and Blood Institute (NHLBI) on the use of the Women's Health Initiative (WHI) biorepository for discovery and validation of biomarkers; the collaboration with the Consortium of Functional Glycomics, funded by National Institutes of Health's (NIH) National Institute of General Medical Sciences (NIGMS) and four carbohydrate research centers funded by NIH's National Center for Research Resources (NCRR).

EDRN unites partners with different research foci, resulting in productive and stable alliances to expedite discovery and development of biomarkers and technologies. For instance, JPL, known for rocket launching, joined forces with EDRN to bring disparate groups of institutions together by creating virtual resources of specimens, biomarkers, tools and technologies, through innovative uses of their informatics infrastructures already validated and proven for the management of planetary data. Another unlikely alliance is NIST and EDRN. NIST is traditionally known for research on physical sciences and standards, not for diagnostics. By joining EDRN, NIST has taken an interest in developing standards for genomics- and proteomics-based diagnostics.

EDRN fosters collaborations with industry. During its inception, EDRN worked with NCI's Technology Transfer Center to develop novel methods for sharing confidential information with industry and EDRN's Technology Resources Sharing Committee developed guidelines for working with industry. EDRN also conducted a workshop on public-private partnerships. Collaborations with the Human Proteome Organization on proteomics and glycomics, the Lustgarten Foundation on pancreatic cancer biomarkers and the Canary Foundation on ovarian cancer markers are yielding results.

EDRN enables alliances of investigators with differing expertise, disciplines and organizational cultures to function as cohesive, integrated groups for the purpose of developing biomarker-based diagnostics. This Network of discovery, validation and epidemiologic centers that place collective goals above individual goals is without peer among academic institutions. Unlike previous approaches in the field, EDRN rewards collaboration and individual skills and thereby is likely to succeed in meeting the new research realities involved in translational research.

EDRN builds standards in study designs for the systematic evaluation of protein profiling for cancer. The Network developed standards of organization and collection for tissue procurement for biomarker studies. Aspects of the standards are recognized as best practices in the field for sharing and dissemination within an informatics network exchange system (*National Biospecimen Network Blueprint* from the Constella Group and the *Case Studies of Human Tissues Repositories: "Best Practices" for Biospecimen Resource for Genomic and Proteomic Era* (Eiseman E., et.al., RAND Corporation)).

The number of peer-reviewed publications by EDRN-funded investigators is an important metric to illustrate progress toward the Network's goals. More than 460 manuscripts have been published by EDRN investigators and program staff in the past 6 years. Seminal articles on proteomics, fusion genes in the prostate and methylation have received wide citations.

When EDRN was created, NCI embarked on a new organizational structure unique to academic science. EDRN created a rigorous peer-review system that ensures that preliminary data—analytical, clinical and quantitative—are of excellent quality. Additionally, the Associate Membership Program is highly productive in offering new technologies and products.

Past, Present, Future

The progression of biomarkers from the discovery phase to the validation phase has been slow to date, reflecting initial challenges with cultural and infrastructural issues.

Without EDRN, research into new biomarkers of early cancer detection and risk would have remained on the periphery of research with a strong, but fragmented laboratory presence and little translational interest among the academic scientific community. But with the Network, a new translational paradigm is defining the organization, approaches and standards by which biomarkers are developed and assessed. The Network's publications, meetings, funding opportunities and infrastructure have fashioned a new environment for cancer prevention research. ■

Part One

Progress and Disease-Specific Development

CHAPTER ONE

Overview

"The EDRN was designed with a very specific and tangible goal in mind. This has not changed since its inception. For this reason, the network is efficient and functions true to its origin. Further, since it is fully functional, there is little effort wasted on operational issues. The operations manual was adopted early and remains a viable document. Under any context, these are remarkable properties, that it was created by a governmental agency is nearly unimaginable. With academic scientists and clinicians working under cooperative agreements, not contracts, to specifically further the goals of the network, not just their personal goals, the arrangement becomes even more unlikely."

Jeff Marks, Ph.D.
Principal Investigator,
EDRN Biomarker Development Laboratory
Duke University Medical Center

IN ITS FIRST 7 YEARS, the Early Detection Research Network (EDRN) evolved from ground-breaking concept to operational success. With a primary mission to discover and scrupulously validate biological markers that signal the earliest stages of cancer (such as pre-malignant lesions, genetic variations and risk indicators) EDRN combines collaborative and multidisciplinary, investigator-initiated projects with a strong administrative and data infrastructure.

In making cancer **biomarkers** of early detection and screening ready for large-scale clinical testing, the Network requires and supports collaboration and information sharing across institutions. Key milestones from inception to the present are described in this chapter.

1997 through 2000: Inception and Inauguration

In 1997, a 20-member Cancer Prevention Program Review Group, seeking a means to revitalize the National Cancer Prevention and Control Program, recommended the concept of EDRN to NCI's Board of Scientific Advisors (BSA) and the National Cancer Advisory Board (NCAB). (See EDRN Initial Report, *Translational Research to Identify Early Cancer and Cancer Risk*, October 2000, http://edrn.nci.nih.gov/docs.)

The concept, developed by the Early Detection Implementation Group, was approved by the BSA on November 13, 1998. A Network was envisioned that would discover and coordinate the evaluation of biomarkers and reagents for risk assessment

Recent Milestones*

March 2003	EDRN Approved for Second 5-Year Cycle
July 2003	Validation Study Launched: SELDI Profiling for Prostate Cancer
August 2003	Gordon Conference on New Frontiers in Cancer Detection and Diagnosis, Andover, NH
September 2003	Launch of the First Clinical Validation Study for Microsatellite Instability as a Biomarker for Bladder Cancer
March 2004	Training Workshop on the Analysis of Proteomic Spectral Data including SELDI/MALDI-TOF-MS Applications; Review of SELDI Phase 1, Seattle, WA
June 2004	Third Annual Scientific Workshop, Bethesda, MD
September 2004	EDRN Outreach Meetings: Breast/GYN Collaborative Group Meeting, New York, NY GI Collaborative Group Meeting, Norfolk, VA GU Collaborative Group Meeting, Houston, TX Lung Collaborative Group Meeting, Denver, CO
January 2005	Gordon Conference on New Frontiers in Cancer Detection and Diagnosis, Santa Barbara, CA
March 2005	Tenth Steering Committee Meeting, Bethesda MD
September 2005	Eleventh Steering Committee Meeting, Seattle, WA
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 Meeting, Rockville, MD
March 2006	Twelfth Steering Committee Meeting and 4th Annual Scientific Workshop, Philadelphia, PA
September 2006	Thirteenth Steering Committee Meeting, Pittsburgh, PA
October 2006	EDRN and Hepatitis B Foundation Workshop, Princeton, NJ
January 2007	Gordon Conference on New Frontiers in Cancer Detection and Diagnosis, Ventura, CA
February 2007	EDRN FDA Education Workshop Bethesda, MD
March 2007	Fourteenth Steering Committee Meeting, Denver, CO
April 2007	AACR Session on Novel Technologies and Validation Challenges, Los Angeles, CA
May 2007	NCI Division of Cancer Prevention Workshop on Cancer Stem Cells as Targets for Cancer Prevention and Early Detection, Bethesda, MD

* See previous reports for earlier milestones.

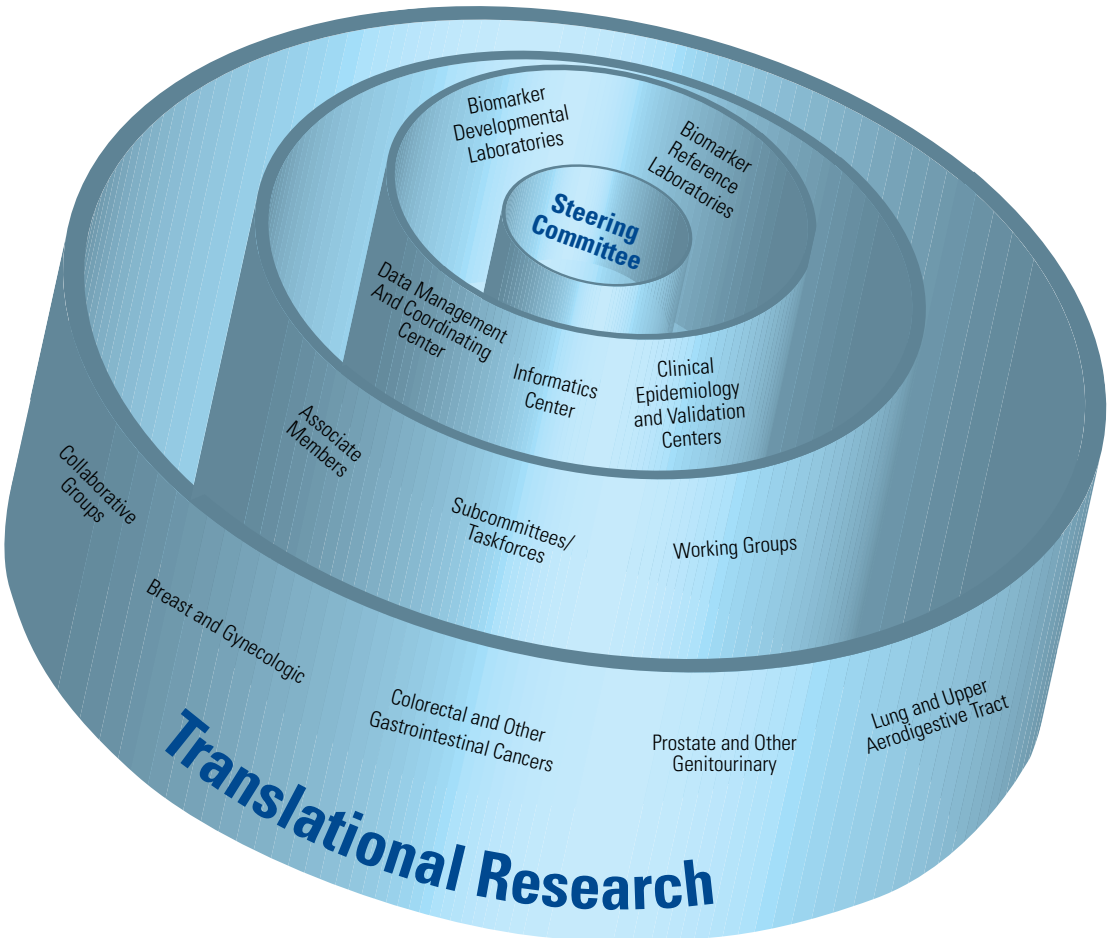
and early detection of cancer in primary organ systems, such as prostate, breast, lung, colorectal and upper aerodigestive tract. To accomplish this vision, the Network would:

- Develop and test promising biomarkers and technologies in institutions with outstanding scientific and clinical expertise;
- Evaluate promising biomarkers for diagnostic predictive accuracy, **sensitivity**, **specificity** and medical benefits;
- Develop molecular and expression markers to serve as background information for subsequent large definitive **validation** studies of detection and screening biomarkers;
- Coordinate academic and industrial leaders in molecular biology, molecular genetics, clinical oncology, computer science, public health and other disciplines to develop high-**throughput**, sensitive assay methods;
- Conduct early phase clinical and epidemiological studies to evaluate the **predictive value** of biomarkers; and
- Encourage collaboration and rapid dissemination of information among participants to aid progress and avoid fragmentation of efforts.

A structure emerged (see Figure 1-1) with working components comprised of laboratories and validation centers and data management centers and two oversight components, a Steering Committee and a Network Consulting Team. The business model for this structure is discussed in Chapter 8.

Figure 1-1. Infrastructure of the Early Detection Research Network

This schematic outlines the EDRN infrastructure for supporting translational research on molecular biomarkers for cancer detection and risk assessment.



The Biomarker Developmental Laboratories (BDLs) were designed to develop and characterize new biomarkers, or refine existing biomarkers, by conducting active **translational research** in the biology of cancer formation. It was expected that discoveries would move from laboratory to clinical and population research settings and that observations from these settings would move back to the laboratory for further refinements as needed.

The Biomarker Reference Laboratories (BRLs) were planned to serve as a resource for both laboratory and clinical validation of biomarkers, in the areas of technology development, standardized assays and methods, refinement and high-throughput operations. BRLs were also responsible for instituting quality control for reagents and technologies.

The Clinical Epidemiology and Validation Centers (CEVCs) were established to conduct and support early phases of clinical and epidemiological research on biomarker applications. Approved projects were soon started to look at a range of issues, including: resources and methods for rapid clinical evaluation of risk and disease biomarkers; defining molecular signatures predictive of neoplastic progression in cervical lesions; clinical utility of certain prostate cancer biomarkers; developing and maintaining a registry of individuals harboring germline mutations for hereditary cancer syndromes; and identifying preneoplastic lesions and early cancer in populations at risk due to environmental and occupational exposures.

To manage the flow of information across the network, the Data Management and Coordinating Center (DMCC) and an Informatics Center, managed by the Jet Propulsion Laboratory (JPL) at the National Aeronautics and Space Administration (NASA) were established. These entities were designed to support statistics, logistics and informatics and develop theoretical statistical approaches for pattern analysis of multiple biomarkers simultaneously. DMCC also coordinates network-wide meetings and conferences and serves as the Coordinating Center for validation studies. (See Margaret Sullivan Pepe, *The Statistical Evaluation of Medical Tests for Classification and Prediction*, Oxford Statistical Science Series Number 28, Oxford University Press, 2003.)

A Steering Committee, comprised of the Network's Principal Investigators and NCI staff, was formed to coordinate the work of the consortium and provide major scientific and management oversight, such as developing and implementing protocols, study designs and general operations.

An *ad hoc* Network consulting team of non-EDRN investigators was instituted to recommend new research initiatives and to ensure Network responsiveness to promising research opportunities. Members of the group have reviewed EDRN as part of the external evaluation process.

Biomarker Reference Laboratories in 2008

These laboratories serve as a Network resource for clinical and laboratory validation of biomarkers.

Principal Investigator	Location
Dan Chan, Ph.D.	Johns Hopkins Medical Institutions
David Chia, Ph.D.	University of California, Los Angeles
Miral Dizdar, Ph.D.	National Institute of Standards and Technology
William E. Grizzle, M.D., Ph.D.	University of Alabama at Birmingham
Karin Rodland, Ph.D.	Pacific Northwest National Laboratory
Sanford Stass, M.D.	University of Maryland School of Medicine

Early Challenges

Establishing and sustaining collaborations while ensuring a smooth flow of discoveries from the laboratory to the clinic were clearly key challenges to the nascent Network. Efforts focused on developing methods for:

- novel approaches to validation studies during the early stages of investigation;
- improved informatics and information flow using new systems for data organization and sharing;
- standardized data reporting by creating a dictionary of neoplastic and pre-neoplastic events and common data elements (CDE) for biomarkers;
- statistical and computational tools; and
- standardized reagents and assays.

Biomarker Development Principles

The Network developed systematic, comprehensive guidelines to develop, evaluate and validate biomarkers. This five-phase approach established both a scientific standard and a roadmap for successfully translating biomarker research from the laboratory to the clinic.

Phase 1 – discovery, involves exploratory study to identify potentially useful biomarkers.

Phase 2 – validation, occurs where biomarkers are studied to determine their capacity for distinguishing between people with cancer and those without.

Phase 3 – determines the capacity of a biomarker to detect preclinical disease by testing the marker against tissues collected longitudinally from research cohorts.

Phase 4 – includes prospective screening studies on biomarker performance in large populations and determines its false referral rate.

Phase 5 – suggests the penultimate period in which large-scale population studies evaluate both the role of the biomarker for cancer detection and its overall screening impact.

Although the Network's focus is mainly on Phases 1 through 3, researchers have welcomed the five-phase structure because it provides an orderly succession of studies that build upon each other to yield an efficient and thorough approach to biomarker development.

Project Prioritization

The Network implemented guiding principles for biomarker validation and used criteria developed by the Review Group to prioritize the first round of proposals for collaborative projects. These principles were:

1. Biologic rationale/strength of hypothesis
2. Strength of design
3. Technical parameters
4. Clinical or scientific impact
5. Portfolio balance
6. Practicality
7. Collaborative strength/team effort

Individual grantees brought to the Network a diverse assortment of potential biomarkers for development. Projects ranged from biomarkers for lung carcinoma and pre-malignancy to cancer risk prediction by mutational load distribution. Some investigators were seeking to detect pre-clinical cancer across a range of organ sites (prostate, liver, ovarian, breast, lung, colorectal) by protein signatures in body fluids using novel technologies such as mass spectrometry and laser capture microdissection. The BRLs set out to validate molecular cytogenetic and automated cytometry assays involving slide-based analysis of chromosomes as a first step to further standards setting.

Collaborative Groups and Associate Memberships

To broaden the opportunities for scientific interactions and coordinated research, Collaborative Groups were formed. These organ-specific research groups were structured to promote information exchange on organ-related biomarkers and to identify research priorities within EDRN.

One major role of the Collaborative Groups was to serve as advisors/liaisons with Associate Members. The Associate Membership component was designed for investigators who are not affiliated with EDRN but wish to join the Network by proposing collaborative studies within its scope and objectives.

Three categories for Associate Membership were established:

- Category A – domestic or foreign investigators who propose to conduct basic or translational research consistent with the priorities of EDRN;
- Category B – domestic or foreign members who contribute to the Network by sharing available technologies and supplying specimens, making available high-risk registries and cohorts and other complementary resources;
- Category C – domestic or foreign corresponding members who are scientists, organizations, clinicians, patient advocates, or ethicists interested in participating in Collaborative Group meetings, workshops and conferences, without EDRN funding.

Profile of the EDRN Associate Membership Program in 2008

- More than 151 applications received since 2000
- Approximately 40 applications approved
- More than 15 diagnostics firms joined as Category C members
- More than 45% of members are new investigators
- More than 60% of Category A members successfully competed for major grants
- Two Associate Members successfully proposed validation studies

2001 to 2003: Meeting the Scientific Challenges

Following the principles of systems biology, in which disciplines like biology, chemistry, computational science and clinical sciences are integrated seamlessly, the Network made strides in meeting the scientific challenges of biomarker research. The first round of proposals for collaborative studies was approved and Steering Committee meetings convened to continue managing the formation of the new Network. (See EDRN Second Report, *Translational Research to Identify Early Cancer and Cancer Risk*, October 2002, http://edrn.nci.nih.gov/docs.)

Discovery Phase

EDRN began actively identifying potential biomarkers and making inroads for testing and evaluating usability in early detection and risk indication. Promising results were attained, such as:

- Lysophosphatidic acid (LPA) was found to be promising as a biomarker and further studies were performed at the discovery laboratories. LPA is elevated in the plasma of women with ovarian cancer including 90% of women with stage I disease.
- A ligand or binding protein for Galectin-3 was pursued at the Great Lakes New England Clinical Epidemiology and Validation Center, which identified the binding protein in circulating blood. Galectin-3 is a protein related to tumor progression and was found to be a hepatoglobin-related protein, present in higher concentrations in patients with colon cancer when compared to those with precancerous polyps or normal subjects.
- A positive finding that androgen receptor-length polymorphism is associated with prostate cancer risk in Hispanic men was made.
- A progression model for bladder cancer was developed.
- The result of an extensive search of gene and protein expression data generated through two-dimensional gel profiles, mass spectrometry, quantitative protein data and gene expression data, found two proteins,

Annexin-1 and Annexin-2, to be candidate biomarkers for lung cancer (*Proc Natl Acad Sci* USA. 2001 98:9824-9). Further validation studies are ongoing.

- Discussions concerning the informatics needs of EDRN were conducted and plans for building the infrastructure began. Prototypes of the EDRN Network Exchange system (ERNE), EDRN Task Management Software, EDRN Statistical Software and the EDRN secure site were produced and tested.

Guidelines Set for Measuring Biomarker Predictive Power

To prepare for the next level of investigation, the Network developed guidelines for statistical design and analysis of nested case-control studies on serially collected blood or tissue specimens. These guidelines, listed below, are used by researchers designing studies to measure the predictive power of a biomarker:

- For clearest interpretation, statistics should be based on false- and true-positive rates, not odds ratios or relative risk.
- To avoid over-diagnosis bias, cases should be diagnosed as a result of symptoms rather than on screening.
- To minimize selection bias, the spectrum of control conditions should be the same in the study and target screening populations.
- To extract additional information, criteria for a positive test should be based on a combination of individual markers and changes in marker levels over time.
- To avoid over-fitting the data, the criteria for a positive marker combination developed in a training sample should be evaluated against random samples from the same study and, if possible, validation samples from another study.

Critical Challenges Faced

The interdisciplinary teams of investigators tackled the critical challenges identified at the beginning: novel approaches to validation studies; advanced informatics and information flow; standardization of reagents and assays and data reporting; and creation of standard statistical and computational tools (see Part II).

New approaches to validation studies were set in motion with preliminary studies in:

- detecting **promoter methylation** as a risk marker;
- chromosomal breakage as a marker of lung cancer susceptibility and early lung cancer detection using **Fluorescence in Situ Hybridization (FISH);**
- mutations in mitochondrial DNA and telomerase activity as early detection markers; and
- **microsatellite instability** as an early detection marker for bladder cancer.

"The EDRN's goals are ambitious and admirably attempt to perform and deliver from both ends of the linear biomedical industries world: to discover new early disease biomarkers and deliver them to the public for use. As if this was not enough, this is to be done across a range of different cancers."

Tim Block, Ph.D.
Principal Investigator
EDRN Biomarker Development Laboratory
Drexel University College of Medicine

Infrastructures were built to improve informatics and information flow across the Network. A public web site and a secure web site contained general and specific information about upcoming events, contacts for institutions and committees, data from collaborative studies and approved validation proposals.

Standardization of data reporting came closer to reality with the development of CDEs required for use at Network sites. In addition, a distribution and computing network, known as the EDRN ERNE, which allows remote access to live databases at each Network site via the secured website, was developed by JPL and the DMCC. ERNE unifies search and retrieval of biospecimen data from all institutions regardless of their location, how data are stored, or the differences in the underlying data models.

Exceptional analytical approaches and methods were developed to generate effective statistical methodologies and computational tools. These incorporated pre-analysis data processing; disease classification; protein biomarker identification; artificial intelligence learning algorithms; **genomic** and **proteomic** data mining; and systems screening.

In collaboration with EDRN's federal partner, NIST, NCI-supported investigators continued during this period to standardize methodologies, refine assays and establish standard reference materials for biochemical, molecular and cytologic assays.

EDRN forged partnerships with the private sector (see Part III). The Network initiated collaborative projects with other NCI-supported programs to leverage shared technology and resources; investigators published abstracts of their work; and liaisons to numerous professional organizations were established.

EDRN Liaisons to Professional and Scientific Organizations

American Association for Cancer Research (AACR): William Bigbee, Ph.D.

American College of Obstetricians and Gynecologists (ACOG): Daniel Cramer, M.D.

American Society for Investigative Pathology (ASIP): Elizabeth Unger, M.D., Ph.D.

American Society of Clinical Oncology (ASCO): Dean Brenner, M.D.

American Society of Preventive Oncology (ASPO): Dean Brenner, M.D.

American Urological Association (AUA): Alan Partin, M.D., Ph.D.

Cooperative Family Registries: John Baron, M.D.

Human Proteome Organization (HUPO): Samir Hanash, M.D., Ph.D.

European Organization for Research and Treatment of Cancer (EORTC): Angelo Paradiso, M.D., Maria Diadone, Ph.D.

Mouse Models of Human Cancers Consortium: Jeffrey Marks, Ph.D.

Pharmaceutical and industrial relations: Wendy Patterson, Esq.

Specialized Programs of Research Excellence (SPORE) Groups: Adi Gazdar, M.D.

Cooperative Groups: Ian Thompson, M.D.

Union Internationale Contre le Cancer (International Union Against Cancer): Michles Bodos, M.D.

2003 to 2004: Network Surges Ahead

NCI supported more than 100 collaborative projects that spanned the organ sites. BDLs investigated biomarker candidates for major organ sites while the first clinical validation study, microsatellite instability as a biomarker for bladder cancer, commenced in September 2003. EDRN's portfolio expanded, its collection of sample sets and reference data sets grew markedly and standard tools and resources were widely utilized. (See EDRN's

Third Report, *Translational Research to Identify Early Cancer and Cancer Risk*, March 2005, http://edrn.nci.nih.gov/docs.)

To make resources available for validation research, a number of technologies were approved and clinical specimens collected and pronounced "open access" for collaborative efforts. In addition, the Network surged ahead in its partnerships with federal agencies through joint projects. Also, a series of workshops, meetings, conferences and collaborative group "town hall" gatherings were held to further cement alliances and share information.

Another unique partnership emerged with the Plasma Proteome Project Initiative of the Human Proteome Organization (HUPO), to evaluate multiple technology platforms, develop bioinformatic tools and standards for protein identification and create a database of the plasma proteome (*Proteomics* August 2005).

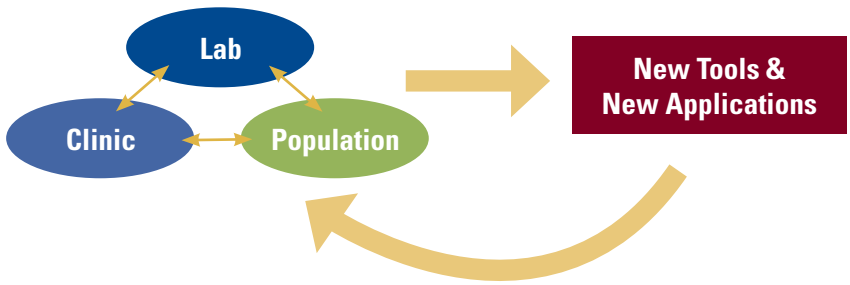
The Network-developed study design for a systematic evaluation of protein profiling, in this case using SELDI-TOF for cancer diagnosis, was published and became a model that can be applied to any other profile-based proteomics platforms. Accordingly, the model was extensively discussed and accepted throughout the research community (*Disease Markers* 2005).

The ERNE knowledge system was deployed to 10 institutions in early 2003, providing a common web-based client interface. Creation of a robust framework called 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.

2005 to 2007: An Investment in Prevention

The NCI's Translational Research Working Group (TRWG) was established in 2005 to evaluate the status of NCI's investments in translational research and chart a vision for the future. TRWG defined translational research as "research that transforms scientific discoveries arising in the lab, clinic or population into new clinical tools and applications that reduce cancer incidence, morbidity and mortality" (see Figure 1-2).

Figure 1-2. Translational Research Paradigm as defined by NCI's Translational Research Working Group



Source: Translational Research Working Group Interim Report to the National Cancer Advisory Board, *Envisioning the Future of NCI's Investment in Translational Research*, June 14, 2006 *(http://www.cancer.gov/aboutnci/trwg/hawk-NCAB.pdf)*

Biomarker Development Laboratories in 2008

These laboratories are responsible for development and characterization of new, or refinement of existing, biomarkers.

Principal Investigator	Location
William L. Bigbee, Ph.D	University of Pittsburgh Cancer Institute
Timothy Block, Ph.D.	Drexel University College of Medicine
Paul Cairns, Ph.D.	Fox Chase Cancer Center
Arul M. Chinnaiyan, M.D., Ph.D.	University of Michigan
Bogdan Czerniak, M.D., Ph.D.	University of Texas M. D. Anderson Cancer Center
Laura J. Esserman, M.D., M.B.A.	University of California, San Francisco
Wilbur Alan Franklin, M.D.	University of Colorado Health Science Center
Adi Gazdar, M.D.	University of Texas Southwestern Medical Center
Samir Hanash, M.D., Ph.D.	Fred Hutchinson Cancer Research Center
Michael Hollingsworth, Ph.D.	University of Nebraska Medical Center
Ann M. Killary, Ph.D.	University of Texas M. D. Anderson Cancer Center
Joshua LaBaer, M.D., Ph.D.	Harvard Institute of Proteomics
Alvin Y. Liu, Ph.D.	University of Washington
Zvi Livneh, Ph.D.	Weizmann Institute of Science
Anna Lokshin, Ph.D.	University of Pittsburgh Cancer Instititute
Jeffrey Marks, Ph.D.	Duke University Medical Center
Martin McIntosh, Ph.D.	Fred Hutchinson Cancer Research Center
Stephen Meltzer, M.D.	Johns Hopkins University
Harvey Ira Pass, M.D.	New York University School of Medicine
Hemant K. Roy, M.D.	Evaston Northwestern Healthcare Research Institute
O. John Semmes, Ph.D.	Eastern Virginia Medical School
David Sidransky, M.D.	Johns Hopkins University
Michael A. Tainsky, Ph.D.	Karmanos Cancer Institute
Richard C. Zangar, Ph.D.	Pacific Northwest National Laboratory

Informatics Center in 2008

The Informatics Center supports EDRN's efforts through software systems development for information management and flow.

Principal Investigator	Location
Daniel Crichton, M.S.	NASA Jet Propulsion Laboratory at the California Institute of Technology

Clinical Epidemiology and Validation Centers in 2008

The Centers conduct clinical and epidemiological research on the medical application of biomarkers.

Principal Investigator	Location
Steven Belinsky, Ph.D.	Lovelace Respiratory Research Institute
Dean Brenner, M.D.	University of Michigan
Daniel Cramer, M.D., Sc.D.	Brigham and Women's Hospital
Paul Engstrom, M.D.	Fox Chase Cancer Center
Henry Lynch, M.D.	Creighton University
Alan W. Partin, M.D., Ph.D.	Johns Hopkins University Department of Urology
William Rom, M.D., M.P.H.	New York University School of Medicine
Martin Sanda, M.D.	Beth Israel Deaconess Medical Center
Ian M. Thompson, M.D.	University of Texas at San Antonio
Elizabeth R. Unger, M.D., Ph.D.	Centers for Disease Control and Prevention

Data Management and Coordinating Center in 2008

The Center is responsible for coordinating EDRN activities by developing a common database for the Network, providing logistic support, conducting statistical and computational research and guiding statistical design and data analyses of validation studies.

Principal Investigator	Location
Ziding Feng, Ph.D.	Fred Hutchinson Cancer Research Center

Program for Rapid, Independent Diagnostic Evaluation (PRIDE):

- The program was modeled on the NCI Rapid Access to Intervention Development (RAID) program, which was designed to assist translation to the clinic of novel anticancer therapeutic interventions, either synthetic, natural product, or biologic, arising in the academic community.
- PRIDE is designed to assist extramural scientists in validating biomarkers and technologies following the device pathway developed by TRWG.
- Initiated in June 2006.
- More than 10 proposals received.
- Three applications are supported.
- Data is shared and analyzed by EDRN DMCC and investigators.

The EDRN has achieved several milestones. The operations manual was proven viable. Guidelines laying out the criteria and sequential study designs for justification of requested resources were provided to investigators. The fully characterized Network provides an unparalleled system of strong scientific collaborations that facilitate high-quality translational research. The infrastructure works to ensure that good biomarkers are promoted without regard to pecuniary interests. The Network's emphasis on inclusiveness allows any scientist, from academia, industry or government to participate in EDRN activities, thus ensuring the best chance for promising markers to become future medical tools.

The Associate Membership Program, along with a newly established Program for Rapid, Independent Diagnostic Evaluation (PRIDE), continues to ensure inclusiveness of stakeholders, biomarkers, technologies and processes all along the EDRN business model. In late 2006, EDRN announced the PRIDE (http://grants.nih.gov/grants/guide/notice-files/NOT-CA-07-003.html), as an administrative means to assist extramural investigators to successfully conduct cross-laboratory validation of biomarkers. Investigators from the diagnostic community were invited to partner with EDRN to develop new standards for methodologies, assays, reagents and tools. This initiative is expected to expand the capacity of existing resources and speed development of diagnostic markers. PRIDE will fill a gap between discovery and clinical application by providing independent evaluation of potential biomarkers developed through various technology platforms and the assays and reagents needed to accelerate them to clinical use. ■

CHAPTER TWO

Breast and Gynecologic Cancers

"Early detection and surgical resection remain mainstays of cancer treatment and the EDRN was, and is, needed to develop the potential of early detection."

Paul Cairns, Ph.D.
Principal Investigator
EDRN Biomarker Development Laboratory
Fox Chase Cancer Center

BREAST AND GYNECOLOGIC cancers are a study in contrasts, from their incidences and detection strategies, to their associated **biomarkers**.

Breast cancer is highly prevalent and has well-established early detection strategies available: mammograms and clinical breast exams. Molecular tests exist to help determine treatment options following breast cancer diagnoses, but the challenge of finding blood tests to detect the disease is daunting. Ovarian cancer is less prevalent than breast cancer, however, it is highly lethal and, thus far, has no approved or marketed early detection tests. (Transvaginal ultrasound and CA-125 are under study in large screening trials.) Recent work within the Early Detection Research Network (EDRN) is leading to a molecular test that is promising and may be widely available in the near future.

Cervical cancer incidence and mortality have been reduced dramatically due to the introduction of the Papanicolaou (Pap) test. Some health care providers are also testing DNA from human papillomavirus (HPV) to determine risk for the disease and the new HPV vaccine may reduce the actual incidence of disease. For this cancer, EDRN targets pre-cancers to improve outcomes and reduce treatments.

EDRN Breast and Gynecologic Cancers Collaborative Group Members

Jeffrey Marks, Ph.D., Chair
Duke University Medical Center

Daniel Cramer, M.D., Sc.D., Co-chair
Brigham and Women's Hospital

Karen Anderson, M.D., Ph.D.
Harvard Institute of Proteomics

Paul Cairns, Ph.D.
Fox Chase Cancer Center

David Chia, Ph.D.
University of California, Los Angeles

Miral Dizdar, Ph.D.
National Institute of Standards and Technology

Richard Drake, Ph.D.
Eastern Virginia Medical School

Paul Engstrom, M.D.
Fox Chase Cancer Center

Laura J. Esserman, M.D., M.B.A.
University of California, San Francisco

Andrew K. Godwin
Fox Chase Cancer Center

Lee Goodglick, Ph.D.
University of California, Los Angeles

Joshua LaBaer, M.D., Ph.D.
Harvard Institute of Proteomics

Anna Lokshin, Ph.D.
University of Pittsburgh Cancer Institute

Martin McIntosh, Ph.D.
Fred Hutchinson Cancer Research Center

Gil Mor, M.D., Ph.D.
Yale University

Christos Patriotis, Ph.D.
National Cancer Institute

Margaret Sullivan Pepe, Ph.D.
Fred Hutchinson Cancer Research Center

Steven Skates, Ph.D.
Brigham and Women's Hospital

Martin Steinau, Ph.D.
Centers for Disease Control and Prevention

Michael A. Tainsky, Ph.D.
Karmanos Cancer Institute

Elizabeth R. Unger, M.D., Ph.D.
Centers for Disease Control and Prevention

Wendy Wang, Ph.D.
National Cancer Institute

Patrice Watson, Ph.D.
Creighton University

Richard C. Zangar, Ph.D.
Pacific Northwest National Laboratory

Breast Cancer

Breast cancers can be detected at very early stages by imaging of the breast. The increase in breast cancer incidence observed over the last 20 years is almost entirely attributable to a detection of ductal carcinoma *in situ* (DCIS) and stage I disease by imaging. Current screening and diagnostic practices can detect lesions that are as small as 1 mm. While there are many cancers missed by these screening approaches, the standard for a successful test must focus on relatively small lesions or those that have yet to become invasive. The costs associated with annual mammogram screenings and tissue biopsies are significantly high and, therefore, the need for additional complementary blood testing strategies is urgent.

Due to the heterogeneous nature of breast cancer, it is unlikely that a single gene or gene product will be useful as a biomarker. This can hinder progress in development of a blood test for clinical application. In this context, several programs to move the field forward have been extensively discussed within EDRN. Novel approaches will be tested in the next year.

Definition of breast cancer: Cancer that forms in tissues of the breast, usually the ducts (tubes that carry milk to the nipple) and lobules (glands that make milk). It occurs in both men and women, although male breast cancer is rare.

Estimated new cases and deaths from breast cancer in the United States in 2007:
New cases: 178,480 (female) and 2,030 (male)
Deaths: 40,460 female, 450 male

One promising approach is the analysis of autoimmunity to the disease using high-**throughput** methods developed by investigators from Karmanos Cancer Institute and Harvard Institute of **Proteomics**. This approach may provide a better handle on the heterogeneity of the disease and the host response to the disease than traditional serum markers.

The second promising area for detection is the creation of an antibody library for breast cancer-specific secreted **glycoproteins**. In a collaboration between Duke University Medical Center and Pacific Northwest National Laboratory, a yeast single-chain antibody library enriched for glycoproteins is being created. This library will greatly increase the number of potential biomarkers for breast cancer.

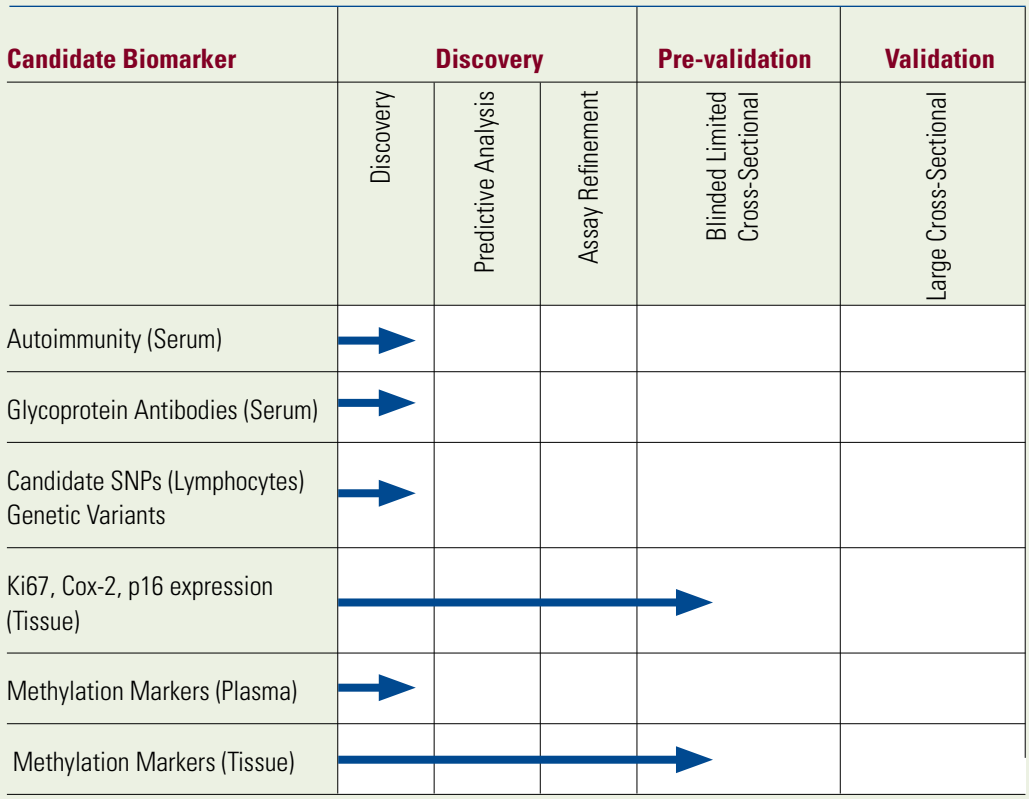
Serum **methylation** markers, while holding promise for breast cancer detection, have significant methodologic and theoretical sensitivity issues that may limit their application in this context.

Two other strategies for improving breast cancer detection are also actively being pursued. In both cases, the central concept is **risk stratification**. As with other cancers, there is a significant hereditary component to breast cancer. Alterations in several genes (*BRCA1* and *BCRA2*) strongly predispose to the disease and the success of genetic testing and subsequent risk management is a paradigm in modern molecular genetics. There are likely to be a number of other genetic variants that are more prevalent in the population but less dramatic in conferring increased risk. Identification of these variants will be incorporated into risk models that should guide future screening and prevention strategies.

Current Early Detection Tests for Breast Cancer

There are currently no biomarker tests to screen for or diagnose breast cancer. The best method of detecting breast cancer early is by regular high-quality mammogram screening and clinical breast exam by a health care provider. Similar to many tests, mammograms have both benefits and limitations. For example, some cancers cannot be detected by a mammogram, but may be found by breast examination. Breast self-exam (BSE) alone has not been shown to reduce the number of deaths from breast cancer. BSE should not take the place of routine clinical breast exams and mammograms.

Candidate Breast Cancer Biomarkers



The second aspect of risk stratification is in the diagnostic setting. In the United States, more than 1 million breast biopsies are performed annually resulting in the diagnosis of about 200,000 cancers. For the frank cancers, subsequent therapeutic steps are reasonably well delineated. However, a large number of women are diagnosed with lesions that are not invasive cancer but may be an early indication of the cancer process. Tissue-based markers that can identify lesions that are likely to progress would be of immediate benefit. Several promising markers have been identified by the University of California, San Francisco and will be further tested and validated in collaboration with EDRN. These two risk-based approaches will integrate into current screening and diagnostic practices and could serve to focus resources on women at the highest risk of developing clinically relevant disease and reduce the morbidity in women with low risk.

EDRN Investigator Honored by DoD

A Department of Defense (DoD) Innovator Award grant, totaling almost $8 million, was awarded in October 2007 to Joe Gray, Director of the Life Sciences Division of the Department of Energy's Lawrence Berkeley National Laboratory (http://www.lbl.gov), and an EDRN Co-P.I. with Dr. Laura Esserman (UCSF).

One of the innovative technologies that is being advanced as part of the Innovator Award, was developed through Dr. Esserman's EDRN "Chair's Challenge" Grant. Via the Chair's Challenge mechanism, Dr. Gray has been collaborating with Dr. Esserman to find better ways to screen women for breast cancer, and to tailor screening to the type of tumors that might develop. Dr. Esserman and colleagues have recently shown data suggesting that current screening is not reducing the risk for women with the most aggressive breast cancers. Other collaborators on the project are from Lawrence Livermore National Laboratories, who are employing a time-of-flight secondary ion mass spectrometer (TOF-SIMS), capable of chemical mapping of breast cells and tumor tissues.

Ovarian Cancer

Early detection of ovarian cancer has been an emphasis for EDRN since its inception. The absence of a useful screening test coupled with the lethality of the disease when it is typically diagnosed at an advanced stage strongly support the potential utility of biomarkers for early detection. The serum tumor marker CA-125 provides a strong foundation to build **multiplexed** tests for the disease.

EDRN investigators have made substantial progress in building and testing marker panels for ovarian cancer. This year, EDRN in collaboration with Specialized Programs of Research Excellence (SPOREs) in ovarian cancer, will perform a head-to-head comparison of a series of blood-based assays that have demonstrated strong predictive value, even in early stage disease. The challenge from this approach will be to narrow the list of candidate markers to those that provide independent value and that can be combined for optimal **sensitivity** and **specificity**. Pre-diagnostic samples from NCI's Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial will yield information on the lead-time before clinical diagnosis of ovarian cancer that these markers will provide. Overall, these robust multi-disciplinary activities should result in clear answers and potential applications for an ovarian cancer diagnostic test within the next two years.

Definition of ovarian cancer:
Cancer that forms in tissues of the ovary (one of a pair of female reproductive glands in which the ova, or eggs, are formed). Most ovarian cancers are either ovarian epithelial carcinomas (cancer that begins in the cells on the surface of the ovary) or malignant germ cell tumors (cancer that begins in egg cells).

Estimated new cases and deaths from ovarian cancer in the United States in 2007:
New cases: 22,430
Deaths: 15,280

Three groups have developed panels of markers that achieve sensitivity (ability to accurately identify people with ovarian cancer) and specificity (ability to accurately identify people without ovarian cancer), in the high 90th percentile in the detection of ovarian cancer in newly diagnosed cases when compared to healthy controls. All three approaches measure the levels of known circulating proteins by either sandwich **enzyme linked immunosorbent assay (ELISA)** or a variation thereof with CA-125 as a key component of each panel of markers. Testing of these panels in additional cohorts revealed a reduced ability to discriminate benign from malignant disease, although they are still highly promising. The next step in validating these marker panels is to test their ability to detect disease prior to clinical

Candidate Ovarian Cancer Biomarkers

Candidate Biomarker	Discovery			Pre-validation	Validation
	Discovery	Predictive Analysis	Assay Refinement	Blinded Limited Cross-Sectional	Large Cross-Sectional
Marker Panels (Serum) – CA-125; MIF-1, prolactin, osteopontin, IGF-2, leptin	→	→	→	→	

diagnosis. Sera from the WHI and the United Kingdom's Ovarian Cancer Screening Trial will be obtained by these groups to test the effectiveness of their markers and algorithms. Within the next 12-18 months, these panels will be tested on pre-diagnostic specimens to determine the sensitivity and specificity and timing of detection of ovarian cancer in a screening situation. These results could pave the way for the phased implementation of such a test in the general population.

Current Early Detection Tests for Ovarian Cancer

To date, no biomarkers are available for screening of ovarian cancer. Studies are ongoing to determine whether routine screening for ovarian cancer with serum markers, such as CA-125, transvaginal ultrasound, or pelvic examinations would result in decreased mortality from ovarian cancer.

Cervical Cancer

Cervical cancer is a testament to the effectiveness of early detection in reducing cancer mortality. Since the introduction of Pap test screening, the incidence of cervical cancer in the United States decreased 70%. While early detection is not primary prevention of cancer, cervical cytology screening programs prevent invasive disease by detecting cancer precursors that can be surgically removed. In addition, the strong etiologic link of HPV with cervical cancer lends itself to a model system for understanding molecular features of other tumors related to infection.

Definition of cervical cancer:
Cancer that forms in tissues of the cervix (the organ connecting the uterus and vagina). It is usually slow-growing and may not have symptoms but can be detected with regular Pap tests (a procedure in which cells are scraped from the cervix and evaluated under a microscope).

Estimated new cases and deaths from cervical (uterine cervix) cancer in the United States in 2007:
New cases: 11,150
Deaths: 3,670

There is a cost that arises from targeting pre-cancers for therapy. Although relatively few of these lesions would progress to invasion, increasingly, even minor abnormalities are treated. Since individual risk cannot be determined, often clinicians and patients prefer to err on the side of over-treatment. In 2007, estimates of new cervical cancers will reach 11,150 with resulting deaths of 3,670. HPV vaccines will not eliminate the need for screening as not all types of HPV-associated cancers are targeted by vaccines. It is estimated that the impact of vaccines on cancer incidence will not occur for 10-15 years after implementation; therefore, screening for vaccine-missed cervical cancers will require even more cost-effective and specific screening tools.

SPOREs, PLCO and EDRN Collaborate to Validate Ovarian Cancer Markers

Three major NCI programs, the Specialized Program of Research Excellence (SPORE), the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial and the Early Detection Research Network (EDRN) are collaborating to validate ovarian cancer biomarkers. Five SPORE sites and two EDRN sites have come together for the first time under the leadership of Nicole Urban, M.D., (SPORE) and Dan Cramer, M.D., (EDRN) in an exemplary effort.

The investigators have identified several putative biomarkers for the detection of ovarian cancers and plan to screen their markers for the ability to detect ovarian cancer in pre-diagnostic and early cases of ovarian cancer specimens collected from the PLCO trial. The hypothesis is that a panel of biomarkers combined into a composite marker will have a lead time sufficient to identify ovarian cancer cases two or more years prior to the time they might be clinically diagnosed.

As a first step, investigators are collectively analyzing their putative ovarian cancer biomarkers in an independent set of ovarian cancer specimens to reconfirm the performance of the markers and to identify a consensus panel of markers. The consensus panel will comprise the biomarkers that are most informative on their own as well as those that are most complementary when used together. The panel will then be used to analyze specimens from women in the PLCO who developed ovarian cancer as well as those that did not. The baseline performance of each marker on its own and in combination will establish a screening rule using pre-diagnostic samples. The screening rule allows the lead-time to be determined.

This collaboration is being coordinated by the EDRN Data Management and Coordination Center (DMCC). If successful, the biomarker panel will likely lead to a clinically approved ovarian cancer screening test.

(See Chapter 6, Validation Studies, Case 3 for more information.)

Current Early Detection Tests for Cervical Cancer

The current number of deaths from cervical cancer reflects an estimated 70% decline from the mid-20th century when the Pap test was first introduced as a screening tool. In addition to the Pap test, some health care providers also test for DNA from HPV, an infection that may increase risk for cervical cancer.

Based on their prior experience with cervical cancer biomarker discovery and validation, the EDRN Cancer Epidemiology and Validation Center modified their study design for biologic sample and data collection. The effort continues to focus on high-risk populations but includes a 2-year longitudinal study designed to provide the follow-up needed to assure precision in disease ascertainment and to evaluate biomarker change in response to therapy. Recruitment was expanded to include HIV-positive women, a more diverse ethnic group and additional women with invasive cervical disease. The biorepository is an EDRN-shared resource and is linked to digitized pathology images producing a virtual slide library permitting web-based pathology of the diagnostic material. Incorporating this new technology allows users of the biorepository to verify case ascertainment.

Numerous molecular biomarkers have been suggested for early detection of cervical cancer but their utility in routinely collected exfoliated cells remains uncertain. EDRN investigators have used **quantitative reverse transcriptase-polymerase chain reaction amplification (qRT-PCR)** to evaluate the expression of 40 candidate genes as markers for high-grade cervical intraepithelial neoplasia (CIN) in exfoliated cervical cells collected at the time of colposcopy. Samples from the 93 women with either CIN3 (the most advanced stage of CIN) or cancer were compared to those from 186 women without disease and matched (1:2) for age, race and high-risk

HPV status. Their diagnostic performance was determined and six markers were found to be promising by exhibiting an area under the curve (AUC) greater than 0.6. (See Figure 3-1 in Chapter 3 for an illustration of this type of diagnostic profile.)

This study supports the concept that exfoliated cervical cells reflect changes in gene transcription that are similar to those found in the biopsy tissue; and because these cells show similar sensitivity and specificity, they perhaps can replace the biopsy to detect CIN. The sensitivity for individual markers was relatively low and a five-gene panel resulted in 60% sensitivity with 76% specificity. Although the results did not indicate superiority of RNA markers for cervical cancer screening, their performance in detecting disease in women referred for colposcopy suggests that the genes and pathways they highlight could be useful in alternative detection formats, or in combination with other screening indicators.

In guiding further work in biomarkers discovery and validation, investigation into the problems of low sensitivity and specificity are being explored using immunohistochemistry to evaluate expression of these markers. In a collaboration with NCI intramural scientists, the performance of a **fluorescent *in situ* hybridization (FISH)** assay for 3q amplification is being evaluated on residual archived liquid Pap samples as those previously evaluated by qRT-PCR for the six promising marker genes. This will allow direct comparison of the assay results. ■

NCI Intramural/CDC Collaboration on Cervical Cancer Biomarkers

Investigators from the Centers for Disease Control and Prevention (CDC) and NCI Intramural research programs collaborate to validate cervical cancer biomarkers for predicting progression. NCI Intramural investigators have found chromosomal gains of 3q using FISH for detecting cervical cancer in an independent study. In the current collaborative study, the performance of chromosomal amplification of 3q by FISH and RNA expression markers (6 marker panel) will be employed in liquid Pap smear samples for predicting the progression of cervical cancer among women with abnormal test results. The specimens were collected in an Interagency Agreement with CDC. The current study, if successful, will reduce the need for repeated colposcopies and enable reduction in the costs for screening cervical cancer.

Candidate Cervical Cancer Biomarkers

Candidate Biomarker	Discovery			Pre-validation	Validation
	Discovery	Predictive Analysis	Assay Refinement	Blinded Limited Cross-Sectional	Large Cross-Sectional
qRT-PCR (6 gene panel) (Exfoliated Cells): CLDN1, MCM5, MCM7, CDC6, MKI67, SHCBP1	→	→	→		

CHAPTER THREE

Colorectal and Other Gastrointestinal Cancers

"The concept of simultaneously testing multiple technologies is starting to be explored in several settings. The idea is to conduct a kind of bake-off in which identical sets of ingredients — strong unbiased specimens — are circulated to laboratories around the country or around the world. In the NCI's Early Detection Research Network we are assessing whether four different serum proteomics technologies can diagnose colon cancer using the same specimens."

David Ransohoff, M.D.
EDRN Associate Member
University of North Carolina, Chapel Hill

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 Early Detection Research Network (EDRN) Gastrointestinal Collaborative Group is working to identify candidate **biomarkers** that will improve patient outcomes in these diseases, through both independent discovery and collaborative work. For each organ site, new biomarkers have been discovered and, in preliminary prevalidation studies, have been shown to be superior to current standards of care. In two circumstances, the newly discovered biomarkers have reached clinical **validation**, an important milestone in the delivery of a new biomarker into clinical use.

EDRN Gastrointestinal Collaborative Group Members

Timothy Block, Ph.D., Chair
Drexel University College of Medicine

Stephen Meltzer, M.D., Co-Chair
Johns Hopkins University

John A. Baron, M.D.
Dartmouth Medical School

Surinder Batra, Ph.D.
University of Nebraska

William Bigbee, Ph.D.
University of Pittsburgh Cancer Institute

Dean Brenner, M.D.
University of Michigan

Robert Bresalier, M.D.
University of Texas M. D. Anderson Cancer Center

David Chia, Ph.D.
University of California, Los Angeles

Ziding Feng, Ph.D.
Fred Hutchinson Cancer Research Center

Radoslav Goldman, Ph.D.
Georgetown University

William Grady, M.D.
Fred Hutchinson Cancer Research Center

Michael Hollingsworth, Ph.D.
University of Nebraska Medical Center

Ann M. Killary, Ph.D.
University of Texas M. D. Anderson Cancer Center

Anna Lokshin, Ph.D.
University of Pittsburgh Cancer Institute

David Lubman, Ph.D.
University of Michigan

Henry Lynch, M.D.
Creighton University

Upender Manne, Ph.D.
University of Alabama, Birmingham

Norman Marcon, M.D.
University of Toronto

Jorge Marrero, M.D.
University of Michigan

Hermant K. Roy, M.D.
Evanston Northwestern Healthcare Research Institute

Robert E. Schoen, M.D., M.P.H.
University of Pittsburgh Cancer Institute

Ying Hsiu Su, Ph.D.
Drexel University College of Medicine

Sapna Syngal, M.D.
Dana-Farber Cancer Institute

Paul Wagner, Ph.D.
National Cancer Institute

Patrice Watson, Ph.D
Creighton University

Colorectal Cancer

Colon cancer is the third most frequently diagnosed cancer in the United States and the third most frequent cause of cancer death. Successful prevention of deaths from colorectal cancer depends on early detection. More widespread use of current screening technologies (fecal occult blood test, sigmoidoscopy, colonoscopy and barium enema) could reduce deaths from the disease, but many people avoid these tests due to their discomfort. Alternate strategies to screen for colorectal cancer could identify those at greatest risk or likelihood of disease versus those who need not submit to an invasive test. EDRN investigators have identified genetic, **epigenetic** and protein biomarkers that correlate with the presence of colorectal cancer using serum, stool and urine.

Definition of colon cancer:
Cancer that forms in the tissues of the colon (the longest part of the large intestine). Most colon cancers are adenocarcinomas (cancers that begin in cells that make and release mucus and other fluids).

Definition of rectal cancer:
Cancer that forms in the tissues of the rectum (the last several inches of the large intestine before the anus).

Estimated new cases and deaths from colon and rectal cancer in the United States in 2007:
New cases: 112,340 (colon); 41,420 (rectal)
Deaths: 52,180 (colon and rectal combined)

The University of Pittsburgh/Johns Hopkins University EDRN Biomarker Development Laboratory (BDL) used **proteomics** focused on the nuclear matrix to identify several serum markers, known as CCSA-2, -3 and -4, that appear to be associated with colon cancer. Using antibodies produced against these markers, investigators are able to differentiate samples from individuals with cancer or advanced adenomas, from samples from normal individuals. Individuals with advanced adenomas are at increased risk for developing colon cancer. Preliminary data show that these biomarkers are more specific (able to accurately identify people with colon cancer) and more sensitive (able to accurately identify people without colon cancer) than current tests. A preliminary validation study is in progress using specimens from the Great Lakes New England Clinical Epidemiology and Validation Center of the EDRN.

Current Early Detection Tests for Colorectal Cancer

Health care providers may suggest one or more of the tests listed below for colorectal cancer screening.

A **fecal occult blood test (FOBT)** checks for hidden blood in the stool. Studies have proven that this test, when performed every 1 to 2 years in people ages 50 to 80, reduces the number of deaths due to colorectal cancer by as much as 30%.

A **sigmoidoscopy** is an examination of the rectum and lower colon using a lighted instrument called a sigmoidoscope. Sigmoidoscopy can find precancerous or cancerous growths in the rectum and lower colon.

A **colonoscopy** is an examination of the rectum and entire colon using a lighted instrument called a colonoscope. Colonoscopy can find precancerous or cancerous growths throughout the colon, including the upper part of the colon, where they would be missed by sigmoidoscopy.

A **double contrast barium enema (DCBE)** is a series of x-rays of the entire colon and rectum. The x-rays are taken after the patient is given an enema with a barium solution and air is introduced into the colon. The barium and air help to outline the colon and rectum on the x-rays. Research shows that DCBE may miss small polyps.

A **digital rectal exam (DRE)** is often part of a routine physical examination. The health care provider inserts a lubricated, gloved finger into the rectum to feel for abnormal areas. DRE allows for examination of only the lower part of the rectum.

Investigators at Evanston Northwestern Healthcare Research Institute are using cutting edge optical technologies to create a device that evaluates the anatomical architecture of the cells lining the colon. Using this technology, a doctor would be able to assess whether or not changes in the overall structure of the cells indicate a risk of developing colorectal cancer. In many cancers, small changes occur in all the tissues exposed to potential cancer-causing compounds, a concept known as field carcinogenesis. They plan to develop a free-standing optical probe that will allow a primary care physician to determine the need for colonoscopy during a digital rectal exam. Spectral markers based on two optics technologies are being employed. In a study of more than 254 people, the spectral-assisted approach was able to detect 100% of the people with cancer (**sensitivity**) and 88% of those without the disease (**specificity**). The test has **positive** and **negative predictive values** of 71% and 100%, respectively, highlighting the ability of this technique to accurately detect patients with adenomas or colon cancer.

The EDRN Great Lakes New England (GLNE) Clinical Epidemiology and Validation Center (CEVC) has a number of ongoing collaborations to discover and validate genomic and proteomic biomarkers for the early detection of colorectal cancer. For example, an EDRN Associate Member at Case Western Reserve University, discovered two proteins, called ColoUp 1 and 2, which can distinguish patients with colon cancer from healthy people without the disease. The GLNE CEVC will support the validation of these markers by supplying blinded specimens for additional testing.

The GLNE is also supporting several sophisticated approaches for the creation of new protein biomarkers or panels of biomarkers for colorectal cancer. The complexity and diversity of proteins derived from clinical specimens present a challenge to conventional proteomics. At the University of Michigan, an EDRN Associate Member developed a new method that simplifies profiles, prior to mass spectral analysis. Specimens from diseased and healthy tissue or sera are resolved, side-by-side, by sophisticated two-dimensional liquid separations (2-D Mass Map). Proteins of interest are then identified by using electrospray **time-of-flight mass spectrometry** (TOF-MS). An analytical test set of sera from 10 individuals with a diagnosis of colorectal cancer, 10 with adenomas and 10 healthy subjects suggests at least 6 proteins that may be altered in abundance as a function of cancer.

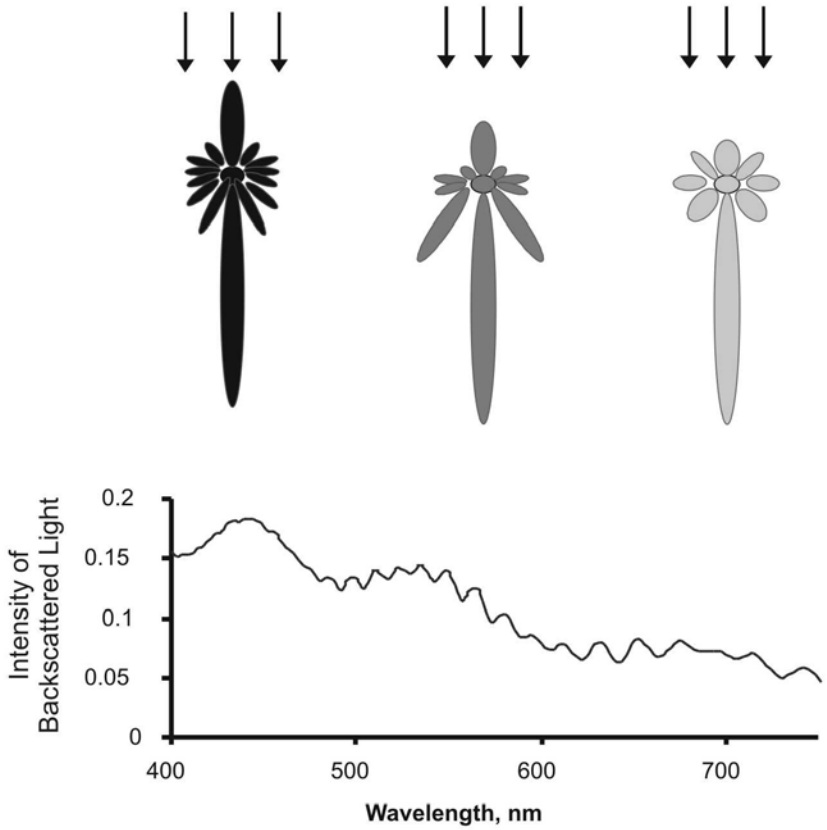
The Drexel University EDRN BDL is collaborating with the GLNE CEVC to determine whether urine can be used as a source of DNA for early colon cancer detection. Their data indicate that mutant K-ras DNA derived from colorectal cancer cells is present in human urine. While they can consistently detect mutant K-ras in urine, this assay does not have sufficient sensitivity and specificity. This issue is being addressed by adding multiple proto-oncogene markers to the assay in collaboration with a commercial partner, Ambergen, Inc.

An EDRN Associate member at the Fred Hutchison Cancer Research Center showed that methylated *CDKN2A* and *MGMT* genes can be detected in fecal DNA from patients with colon adenomas (**Methylation** Marker Panel). Overall, methylation of at least one of the candidate genes was detected in the fecal DNA from 57% of patients with adenomas.

Although less sensitive than colonoscopy, the current gold standard for colon cancer screening, these methylated genes have the potential to be cost-effective screening markers considering the current price for the molecular marker assay commercially available for colorectal cancer screening.

Figure 3-1. Optical Probe for Colon Cancer Screening

Through collaboration between clinicians/biologists at Evanston-Northwestern Healthcare and Biomedical Engineers at Northwestern University, the EDRN has been testing the ability of powerful new optical technologies such as four-dimensional elastic light-scattering fingerprinting (4D-ELF) to cancer screening. The light scattering information, harnessed by spectral biomarkers, is exquisitely sensitive to the nanoscale architectural changes of cells. This provides a highly accurate and practical means of detecting the genetic/epigenetic changes in field carcinogenesis that occur in microscopically normal epithelium. In a study of 250 patients, spectral markers obtained from the endoscopically normal rectal mucosa had a 100% sensitivity and 89% specificity for predicting the presence of advanced neoplasia anywhere in the colon. Ongoing studies employing a fiber optic probe will aim to validate this minimally intrusive rectal test as a pre-screen, thus enabling rational tailoring of colon cancer screening regimens. Moreover, through the Network collaborative process, spectral markers are being tested for risk stratification of a number of other malignancies including gastric, biliary and lung.



These arrows represent different wavelengths of incident light. The scattering angle and intensity for a particular wavelength of light are determined by the size and structure of the scattering particle. These three objects give rise to different light scattering diagrams.

Source: EDRN investigators at Evanston-Northwestern Healthcare and Northwestern University

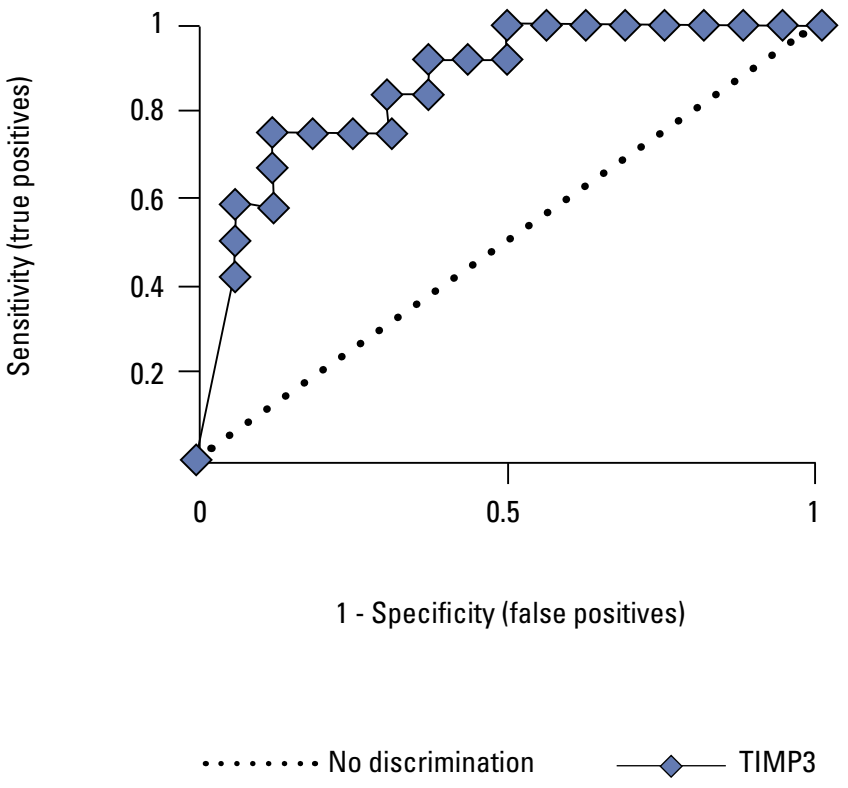
Candidate Colorectal Cancer Biomarkers

Candidate Biomarker	Discovery			Pre-validation	Validation
	Discovery	Predictive Analysis	Assay Refinement	Blinded Limited Cross-Sectional	Large Cross-Sectional
CCSA-2, 3 and 4 (Serum)	→	→	→	→	
Spectral Markers (Tissue)	→	→	→	→	
ColoUp 1 and 2 (Serum)	→	→	→	→	
2D Mass Map (Serum)	→	→	→		
K-Ras (Urine)	→	→	→	→	
Methylation Marker Panel (Stool)	→	→	→		
TIMP-3 Methylation (Tissue)	→	→	→	→	
SELDI Profile (Serum)	→	→	→		
MALDI Profile (Serum)	→	→	→		
K-Ras (Stool Guiac)	→	→	→	→	
Flat adenoma (Tissue)	→	→			
GOS (Stool)	→	→	→	→	
Galectin-3 Lig (Serum)	→	→	→	→	
TIMP-1 (Serum)	→	→	→	→	

Approximately 50% of patients above the age of 60 have polyps discovered on colonoscopy. Current practice is to have repeat colonoscopies at 3-year intervals following the initial polypectomy. Many of these follow-up procedures do not show recurrent adenomas. A means of identifying those patients who are most likely to have recurrent polyps would be useful to reduce the number of negative follow-up colonoscopies and to identify patients in whom polyp recurrence is most likely. EDRN investigators at Johns Hopkins University are exploring the use of DNA methylation markers to predict polyp recurrence. These investigators analyzed the methylation status of 15 candidate genes in polyps that were removed during colonoscopy from 53 patients. Each of these patients had follow-up colonoscopies performed. The EDRN investigators found that the methylation status of several of these genes in the polyps removed during the first colonoscopy could be used to predict those patients who would have polyps during their follow-up colonoscopies. Results with TIMP-3 methylation in predicting polyp recurrence at 24 months are shown in Figure 3-2. A panel of methylation markers is currently being created from studies with an expanded cohort of patients. These prediction biomarkers may stratify patients into follow-up groups to determine the necessity and appropriate interval for follow-up colonoscopies.

Figure 3-2: Ability of TIMP-3 Test to Identify Likelihood of Polyp Recurrence at 24 Months

The dotted line would indicate the test had no ability to predict recurrence, while a "perfect" test would reach 1 in sensitivity and go straight across.



Source: EDRN investigators at Johns Hopkins University

Hepatocellular Carcinoma/Primary Liver Cancer

Primary hepatocellular carcinoma (HCC) is fifth in cancer incidence worldwide and the third leading cause of cancer death. It is also the fastest growing, in incidence, in the United States with a 5-year survival rate of less than 5%. The high mortality associated with HCC is primarily due to the advanced stage of disease at initial diagnosis when therapy is not successful. Infection with hepatitis B or hepatitis C virus (HBV and HCV) is responsible for at least 80% of all HCC. Since patients with cirrhosis (with or without HBV or HCV infection) are at significantly increased risk of developing HCC, surveillance of a clinically identifiable population is realistic and logical. Thus, screening for HCC focuses on patients with cirrhosis. By analyzing existing biomarker candidates as well as through its own proteomic program, EDRN identified three new leading biomarker candidates, two of which are the focus of a large multi-site validation trial.

Definition of liver cancer:
Primary liver cancer is one that forms in the tissues of the liver.

Estimated new cases and deaths from liver and intrahepatic bile duct cancer in the United States in 2007:
New cases: 19,160
Deaths: 16,780

Primary liver cancer incidence rates have increased 2.2% per year from 1995 to 2004, the most recent year statistics are available.

The level of alpha-fetoprotein (AFP) in blood is currently used in the diagnosis of HCC, with a sensitivity ranging from 39% to 62% and a specificity from 56% to 80%. This results in a positive **predictive value** ranging from 16% to 35%, missing many early stage HCC and giving many false-positive results.

At the University of Michigan, an EDRN Associate Member, conducted a case-control study to compare the accuracy of circulating AFP levels versus levels of des-gamma-carboxy prothrombin (DCP) as a biomarker of HCC. DCP was found to have excellent sensitivity in HCC detection (89%) and specificity (95%), significantly better than the performance of AFP in the same patients. These results have been validated in a multi-center trial, using blood collected from patients with early stage HCC. (See Chapter 6, Validation Studies, Case 1.) The ability of AFP-L3% to detect early stage HCC was also evaluated. AFP-L3% is a form of AFP recently approved by the FDA to help doctors determine the chances that a patient will advance to liver cancer in the next two years so the patient can be appropriately managed and treated.

Current Tests to Diagnose Liver Cancer

There are no early detection tests for liver cancer. However, the following tests are FDA-approved for use in the diagnostic process:

alpha-fetoprotein (AFP)

A protein normally produced by a fetus. AFP levels are usually undetectable in the blood of healthy adult men or women (who are not pregnant). An elevated level of AFP suggests the presence of either a primary liver cancer or germ cell tumor.

AFP-L3%

A subgroup of AFP with a particular sugar structure alteration that is known to be highly produced by liver cancer cells. When this form exceeds 10% of the total AFP, the test is considered positive.

At Drexel University an EDRN team, working with The Hepatitis B Foundation, used new **glycomics** techniques to discover several novel **glycoprotein** biomarkers of liver disease. Their work focused on the subset of polypeptides that contain the sugar fucose. This group identified more than 50 fucosylated

serum proteins whose levels are elevated in the serum of people with HCC. One protein, GP73, normally resides inside the cell on the Golgi apparatus. At present, GP73 levels have been measured in samples from more than 700 individuals and were shown to be more sensitive than AFP in detecting both early and late stage HCC. In distinguishing early stage HCC from cirrhosis, GP73 had a sensitivity of 62% compared with 25% for AFP. The assay was adapted to specifically detect fucosylated GP73, which significantly improved its performance: fucosylated GP73 was elevated in 90% of those with HCC and none of those with cirrhosis. HCC is more closely associated with **fucosylation** than protein biomarker levels, as the fucosylated forms of other serum proteins, such as hemopexin and kinnogen, also had sensitivities of more than 80% with specificities more than 90%. Once the assays for these fucosylated proteins are fully developed, the results will be validated using the specimens collected for the EDRN DCP Validation Trial.

Glycomics for Biomarker Discovery

Research is showing that the glycosylation of proteins may vary with disease state. That is, the same protein from malignant and non-malignant tissues may occur in different and characteristic glycoforms. EDRN scientists at Drexel University have developed methods by which N-glycans derived from certain serum glycoproteins as a function of liver disease can be analyzed and, the glycans that correlate with hepatocelluar carcinoma (HCC) can be identified. The approach can be used for any disease, but beginning with an animal model, these scientists identified glycans and the glycoproteins to which they are attached that were associated with HCC. An assay to detect the most prominently associated protein, called GP73, was developed and now more than 1,000 samples from people have been tested in blinded studies. GP73 and its fucosylated glycoform, are proving to outperform the standard of care, alpha-fetoprotein, in detection of early stage cancer. This represents an important proof of concept as well as a delivery of superior early detection markers of liver cancer.

Treatment decisions for people with cirrhosis are often based upon the degree of fibrosis or scarring in their liver. A blood test to determine the degree of liver fibrosis would permit treatment decisions to be made without further invasive procedures. Investigators at Drexel University found that the major fucosylated polypeptide in the circulation of those with a diagnosis of cirrhosis is a distinct immunoglobulin. Based on these findings, a simple **enzyme linked immunosorbent assay (ELISA)** to detect this form of immunoglobin was developed that can distinguish fibrosis stage F3 and greater from stage F2 and less, with a positive predictive value of 97%. If validated, this test could be used to determine treatments.

An EDRN Associate Member at Georgetown University is analyzing the low molecular weight proteins found in the serum of liver cancer patients. A computational method was used to select six marker candidates for classification of HCC. The performance of these candidates was assessed by examining sera of 78 HCC cases and 72 age- and gender- matched cancer-free controls recruited from an Egyptian population. A combination of the six markers using a **matrix-assisted laser desorption (MALDI)** profile achieved 100% sensitivity and 91% specificity. These results await verification using specimens from American patients.

Barrett's Esophagus and Esophageal Adenocarcinoma

Despite advances in surgical techniques and multimodal therapy, the 5-year survival rate for esophageal cancer remains dismal at 5% to 15%. Advanced stage of disease at initial diagnosis and high rates of recurrence contribute to this low survival. Developing and refining methods for early cancer detection is a key to improving survival in this deadly disease.

Candidate Hepatocellular Cancer Biomarkers

Candidate Biomarker	Discovery			Pre-validation	Validation
	Discovery	Predictive Analysis	Assay Refinement	Blinded Limited Cross-Sectional	Large Cross-Sectional
Des-gamma Carboxy Prothrombin (DCP) (Serum)					
AFP-L3% (Alpha-fetoprotein) (Serum)					
GP73 (Serum)					
f-GP73 (Fucosylated-GP73) (Serum)					
Fucosylated (f)-kinnogen (Serum)					
Fucosylated (f)-hemopexin (Serum)					
Fucosylated polypeptide for fibrosis (Serum)					
MALDI Profile (6 protein peaks) (Serum)					

Definition of esophageal cancer:
Cancer that forms in tissues lining the esophagus (the muscular tube through which food passes from the throat to the stomach). Two types of esophageal cancer are squamous cell carcinoma (cancer that begins in flat cells lining the esophagus) and adenocarcinoma (cancer that begins in cells that make and release mucus and other fluids).

Estimated new cases and deaths from esophageal cancer in the United States in 2007:
New cases: 15,560
Deaths: 13,940

Definition of Barrett's esophagus:
A condition in which the cells lining the lower part of the esophagus have changed or been replaced with abnormal cells that could lead to cancer of the esophagus. The backing up of stomach contents (reflux) may irritate the esophagus and, over time, cause Barrett's esophagus.

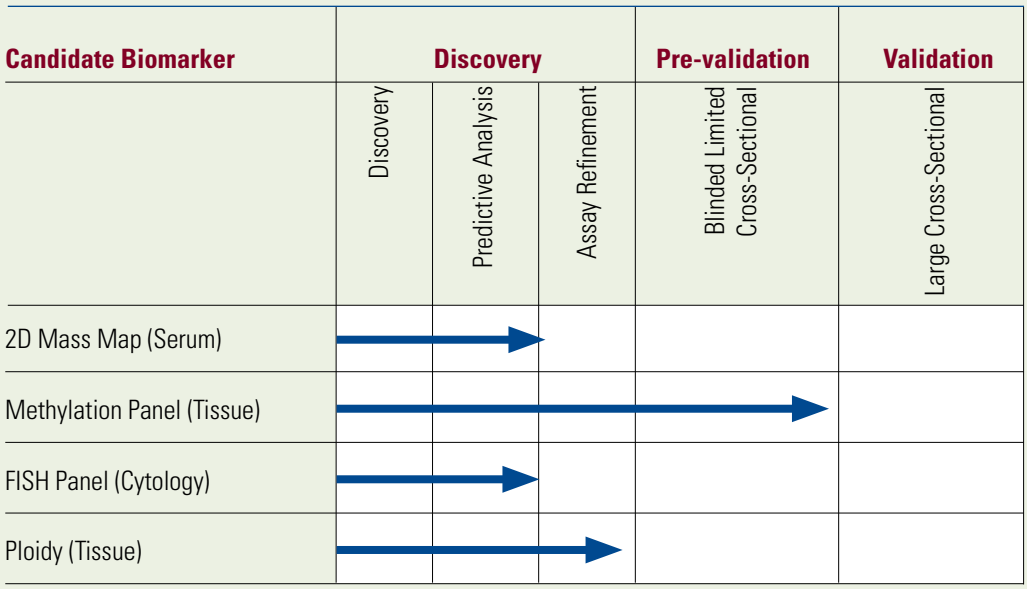
There are no early detection tests for esophageal cancer.

Chronic reflux of acidic gastric contents can cause gastroesophageal reflux disease or GERD. Long-term GERD, in turn, can cause Barrett's esophagus, a premalignant condition that increases a patient's risk of developing esophageal adenocarcinoma. Because of this increase in cancer risk, patients with a known diagnosis of Barrett's esophagus undergo endoscopic surveillance at regular intervals, usually every two to three years. Patients may undergo these surveillance endoscopies for the rest of their lives, sometimes submitting to as many as ten in a lifetime. However, most patients with Barrett's esophagus do not progress to cancer and a biomarker test to predict those likely to progress could reduce the number of endoscopies and improve surveillance.

An EDRN investigator at Johns Hopkins University developed a three-tiered risk model that incorporates both **epigenetic** (methylation of tumor suppressor genes) and clinical parameters to improve the efficiency of Barrett's esophagus surveillance. As progression-free survival differed significantly among the three risk groups, clinicians may be able to base the frequency of endoscopies on an individual patient's risk calculated using these epigenetic and clinical parameters. A related project also involves analyzing levels of methylated DNA in plasma from patients with Barrett's esophagus or esophageal adenocarcinoma. Among 24 patients with esophageal adenocarcinoma studied to date, 70% had hypermethylated HPP1 in their blood, compared with only 13% of control subjects. Thus, DNA methylation in sera may be useful for early detection of esophageal adenocarcinoma and as prognostic or recurrence biomarkers for this deadly disease.

EDRN investigators at the University of Michigan are working with a number of collaborators to develop and validate both proteomic and **genomic** biomarkers for esophageal adenocarcinoma. Their collaborators at the Mayo Clinic have performed a pre-validation study of a panel of **fluorescence *in situ* hybridization (FISH)**-based biomarkers to detect high-grade dysplasia and adenocarcinoma using esophageal cytology. These FISH probes examine alterations (loss or gains) in specific genetic loci associated with the progression of Barrett's esophagus to esophageal adenocarcinoma. Abbott Molecular, Inc., the sponsor of this study, is ready to collaborate with EDRN to perform a multi-site study. In another collaboration, EDRN investigators at UCLA will evaluate the use of ploidy (numbers of chromosomes in a cell) as a predictor for progression of esophageal cancer.

Candidate Esophageal Cancer Biomarkers



Pancreatic Cancer

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.

Definition of pancreatic cancer:
A disease in which malignant (cancer) cells are found in the tissues of the pancreas.

Estimated new cases and deaths from pancreatic cancer in the United States in 2007:
New cases: 37,170
Deaths: 33,370

Commonly used imaging methods, such as endoscopic ultrasound, abdominal CT scan, or MRI, are inadequate for the detection of early stage pancreatic cancer. CA 19-9 is presently the most widely used serum marker for pancreatic cancer, but as a screening test in an asymptomatic population, its positive predictive value is below 1%. EDRN investigators are actively exploring both genomic and proteomic markers to improve the ability to detect early stage pancreatic cancers.

At the University of Nebraska EDRN investigators are working to improve the utility of CA 19-9 by adding a test to determine the mucin protein to which the CA 19-9 carbohydrate antigen is attached. This proposal is based on recent discoveries about the different mucin core proteins expressed by different types of cancers. This group found that levels of expression of specific mucin genes are increased in pancreatic cancer tissues and that an antibody against one of these mucin proteins can detect 91% of pancreatic cancer in endoscopic ultrasound-guided fine needle aspirate samples. This group is currently working to develop serum assays for these mucin proteins.

Scientists at the University of Texas M. D. Anderson Cancer Center are taking a targeted approach to identify biomarkers for early detection of pancreatic cancer by focusing on abnormal genetic pathways in pancreatic cancer. They have identified a number of genes that are consistently differentially expressed in pancreatic cancer

and are examining these genes as candidate biomarkers. Among them, *Sel-1L* is of particular interest as at least one form encodes a secreted protein. Ductal epithelial associated ring chromosome 1 (*DEAR1*), a gene that maps into a region of high frequency loss of heterozygosity in sporadic breast and pancreatic cancer, is another candidate gene discovered by this laboratory. *DEAR1* methylation assays as well as *DEAR1* mutation assays are being developed.

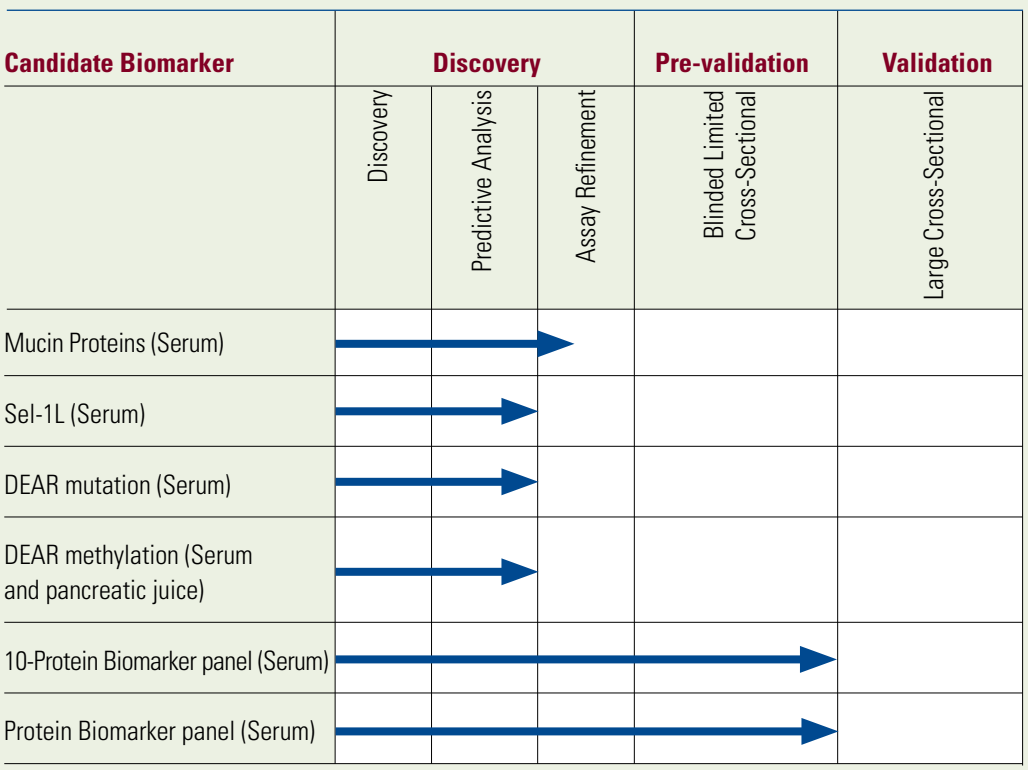
There are no FDA-approved early detection tests for pancreatic cancer.

The CA 19-9 assay is used as a tumor marker when pancreatic cancer is diagnosed, but is not a good diagnostic test. The test measures the level of CA 19-9 in the blood from both cancer cells and normal cells. Higher than normal amounts of CA 19-9 in the blood can be a sign of gallbladder or pancreatic cancer or other conditions.

A protein array system to analyze blood samples from patients with pancreatic cancer is being exploited at the University of Pittsburgh Cancer Institute. These investigators found marked differences in the expression of many proteins in patient samples compared with those from controls. This led to the development of a 10-biomarker panel that distinguishes pancreatic cancer patients from healthy controls with a sensitivity of 87% and a specificity of 98%. This panel specifically recognized patients with pancreatic cancer and excluded patients with other cancers, including lung, esophageal, head and neck, ovarian, breast, endometrial and melanoma.

Another EDRN team at the Fred Hutchinson Cancer Research Center developed a panel of protein biomarkers in serum that can distinguish patients with pancreatic cancer from those with pancreatitis (inflammation of the pancreas) with nearly 95% sensitivity and specificity. Biomarker panels developed by both EDRN teams are very promising and plans are in progress to validate those using larger numbers of specimens, especially from early stage disease, collected from multiple sites. Clues to discovery of these biomarkers came from the investigators' study on mouse proteomics. ■

Candidate Pancreatic Cancer Biomarkers



CHAPTER FOUR

Lung and Upper Aerodigestive Cancers

"Our research provides solid evidence on universal involvement of forerunner genes in human carcinogenesis. In fact, they are used in methylation panels for other organs such as lung …. In general the forerunner gene may represent novel therapeutic preventive targets and early detection markers for other cancer types. EDRN has provided critical funding and collaborating platforms for this PI not available via other funding mechanisms. The goals of EDRN are ideal for our research on early phases of human carcinogenesis and markers development for early cancer diagnosis."

Bogdan Czerniak, M.D., Ph.D.
Principal Investigator
EDRN Biomarker Development Laboratory
University of Texas M. D. Anderson Cancer Center

LUNG CANCER CONTINUES to be the most lethal cancer in the United States. The reasons for this high mortality are advanced stage at diagnosis, the biological aggressiveness of the tumor and its resistance to standard radiation and chemotherapy. The 5-year survival rate after diagnosis remains discouragingly low at only 15%. If lung cancer is detected early, survival is much improved, up to 90% 5-year survival. Computerized tomography (CT) can detect a large number of non-specific lung nodules that require follow-up scans to determine growth rate or invasive procedures to establish a diagnosis. This process causes anxiety, significant financial burdens to the patient and the health care system and exposes patients to potentially harmful amounts of radiation. As the majority of these nodules are benign, a **biomarker** or panel of biomarkers that can distinguish benign nodules from cancer are needed.

EDRN set goals to identify and validate non-invasive biomarkers in blood or sputum for the early detection of lung cancer, which could be combined with CT scanning or other imaging methods. Smoking is the leading risk factor for lung cancer; however a second significant, but less common, risk factor is asbestos exposure. EDRN biomarker discovery efforts for lung cancer take these causative agents into account. In addition, EDRN supports diagnostic studies for early detection of mesothelioma, a malignancy that is almost always associated with exposure to asbestos.

EDRN Lung Cancer Collaborative Group Members

William Rom, M.D., M.P.H., Chair
New York University School of Medicine

Adi Gazdar, M.D., Co-Chair
University of Texas Southwestern Medical Center

David Beer, Ph.D.
University of Michigan

William Bigbee, Ph.D.
University of Pittsburgh Cancer Center

Steven Belinsky, Ph.D.
Lovelace Respiratory Research Institute

Wilbur Franklin, M.D.
University of Colorado Health Science Center

Lee Goodglick, Ph.D.
University of California, Los Angeles

Samir Hanash, M.D., Ph.D.
Fred Hutchinson Cancer Research Center

Karl Krueger, Ph.D.
National Cancer Institute

Carolyn Muller, M.D.
University of New Mexico

Zvi Livneh, Ph.D.
Weizmann Institute of Science

Harvey Pass, M.D.
New York University School of Medicine

David Sidransky, M.D.
Johns Hopkins University

Lynn Sorbara, Ph.D.
National Cancer Institute

Mark D. Thornquist, Ph.D.
Fred Hutchinson Cancer Research Center

Elizabeth Unger, Ph.D.
Centers for Disease Control and Prevention

Lung Cancer

Gene **promoters** play a central role in gene regulation. Promoter methylation is a process by which cancer cells add methyl groups to cytosine bases in promoters thereby turning off the gene. These methylated genes become inactivated and their vital cellular pathways are altered, often increasing the malignant potential of the cells. In addition, promoter methylation is readily detectable by **quantitative** molecular techniques based on **polymerase chain reaction (PCR)** amplification. The identification of multiple methylated sites on promoters is emerging as a powerful marker of a cell's transformation from normal to cancer.

EDRN investigators have extensive experience in the identification of new methylated markers in lung cancer. These discovery efforts have led to the testing of various methylated promoters as markers of lung cancer by testing sputum and cell-free DNA in blood. One interesting study involving collaboration between Johns Hopkins University and New York University investigators allowed comparisons of current high-profile imaging techniques (spiral CT scanning) with emerging methylated markers in the corresponding clinical samples. The Clinical Epidemiological and Validation Center (CEVC) at New York University is using spiral CT scanning to screen over 1,000 people at high risk for developing lung

cancer (see Figure 4-1). These people are heavy smokers or were occupationally exposed to carcinogenic agents. Studies show that CT-scan screening can increase detection of early lung cancer seven-fold compared to the chest x-ray, with the greatest improvement in determining Stage I and II adenocarcinoma. In these early stages of lung cancer, surgical therapy can allow up to 90% survival for four or more years. The objective of the National Lung Screening Trial (NLST) is to determine if mortality can be affected by spiral CT screening.

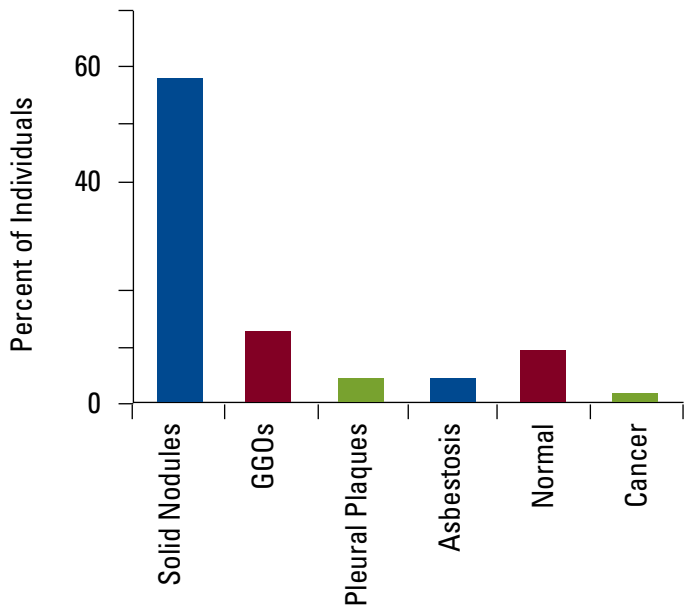
Definition of lung cancer:
Cancer that forms in tissues of the lung, usually in the cells lining air passages. The two main types are small cell lung carcinoma and non-small cell lung carcinoma. These types are diagnosed based on the cellular morphology under a microscope.

Estimated new cases and deaths from lung cancer (non-small cell and small cell combined) in the United States in 2007:
New cases: 213,380
Deaths: 160,390

Figure 4-1. CT Scan Screening for Lung Cancer

CT-screening detects several kinds of tissue changes in lungs (see figure below). In over half of high-risk (>20 pack-years) smokers, noncalcified nodules 4-8 mm in size are found and approximately another 10% of subjects have ground-glass opacities (GGOs). These nodules and GGOs require follow-up to determine if they are cancer. EDRN investigators are exploring different avenues to complement CT-screening by identifying biomarkers in blood or sputum applicable to the diagnosis of these suspicious abnormalities. The importance of such biomarker tests is that clinical decisions can be made by non-invasive means to establish early stage cancer or prevent unnecessary surgeries, minimize stress and avoid prolonged follow-up to patients with benign nodules.

Profile of subjects entering spiral CT screening at New York University CEVC



Source: CEVC at New York University

DNA in plasma and sputum samples from patients with lung abnormalities detected upon CT scan was examined for aberrant methylation of four gene promoters (*CDH1, RASSF1A, p16 a*nd *MGMT*). The patients were divided into three groups based on characteristics of the abnormalities detected: nodules, ground-glass opacities (GGOs) and cancerous tumors. Plasma and sputum DNA from age-matched nodule-free individuals were used as controls. In plasma, 30% of patients with nodules, 32% of patients with GGOs and 48% of patients with cancerous tumors showed methylation of at least one gene while only 4% of control patients showed methylation. In sputum, 18% of patients with nodules and 41% of patients with GGOs showed methylation whereas no control subjects exhibited methylation.

Methylation of at least one marker in either the patient's plasma or sputum was observed in 6% of controls and 40% of patients with abnormal findings. Promoter hypermethylation of a panel of genes increases the likelihood of detecting such changes in plasma and sputum from patients with abnormal spiral CT findings in the lung. An extended set of genes is being investigated including *APC, Cyclin D2,* β*-Catenin, FHIT, DNA Methyltransferase (DNA MTase), Calcitonin-Related Peptide* and *Deleted in Colorectal Cancer (DCC).* Three laboratories will independently evaluate these methylation markers for reproducibility and performance. Longitudinal follow-up of the present cohort will determine the risk of developing neoplastic lung disease for patients with abnormal CT findings who do not exhibit methylation of these genes.

Similar studies are being conducted by EDRN investigators at the Lovelace Respiratory Research Institute to determine whether a panel of genes can be identified whose methylation in sputum will predict early lung cancer. This hypothesis received considerable support through recent findings from a nested, case-control study within the Colorado High-Risk Smokers cohort. The findings showed that the concomitant methylation of three or more of the six genes in the panel was associated with a six-fold increased risk for lung cancer. A **sensitivity** and **specificity** of 64% were seen for identifying incident lung cancer cases three to eight months prior to clinical diagnosis. Additional genes are under evaluation to increase the sensitivity and specificity of the methylation test in sputum. Other studies of methylation in sputum are being conducted in two high-risk cohorts with longitudinal follow-up: a veterans male cohort and the Lovelace Smokers cohort that is predominantly female. Cross-sectional and nested, case-control studies are in progress.

A third study based on diagnosis of current lung cancer involves collaboration between EDRN investigators at the University of Texas-Southwestern with a group from the Netherlands. They have developed novel methods for sputum collection and processing and have amassed over 600 specimens from cancer patients and heavy smokers without cancer, often with extensive follow-up data on these patients. In two preliminary blinded experiments, a panel of only two marker genes readily identified lung cancers at specificity and sensitivity values exceeding those of conventional cytology by two to three times. These promising data are now being pursued by testing a larger panel of methylation markers in 40 specimens from Lovelace. Pending the success of this study, a large **validation** study will be proposed using specimens collected from multiple EDRN sites and by the Dutch group with methylation analysis to be performed independently at laboratories in the United States and the Netherlands.

In a related study a collaboration between New York University investigators with researchers at the Fox Chase Cancer Center have shown that plasma levels of S-adenosylmethionine, the methyl donor for DNA methylation, are markedly increased in patients with lung cancer compared with smokers with normal CTs. These findings suggest that plasma S-adenosylmethionine may serve as an additional biomarker for malignancy; the increase in S-adenosylmethionine may be related to increased gene methylation.

One of the most consistent properties of lung cancers is their high level of chromosome instability that is reflected in imbalance of chromosome copy number and widespread structural abnormalities. Researchers at the University of Colorado have found **clonal changes** that precede the development of carcinoma in the central airway. The most frequent are abnormalities in chromosome number (usually chromosomes 5, 7, 8 and 18) that result from mis-segregation during cell division. Chromosomal instability associated with the development of spreading mutant clones in the airways was found in approximately 40% of high-risk smokers who do not have lung carcinoma. In addition, clonal changes were identified in benign epithelium from approximately half of the patients whose tissues have been removed for lung carcinoma. No abnormalities were observed in never-smoking controls. In collaboration with Abbott Laboratories, **fluorescence *in situ* hybridization (FISH)** probes are being developed that will recognize all of these lesions.

In search of other biologic markers, EDRN investigators at Johns Hopkins and the National Institute of Standards and Technology (NIST) have turned to mutations in mitochondria, the energy factories of cells. Reports from several laboratories have found somatic mutations of mitochondrial DNA in most human tumors. Efficient efforts of mutation detection were hampered by the 16,000 mitochondrial DNA bases that must be tested. This barrier was recently overcome by the development of a sensitive mitochondrial DNA sequencing chip called the Mito Chip. This chip was exploited to investigate whether mitochondrial mutations can be detected from non-invasively collected bodily fluids (sputum or bronchoalveolar lavage) in lieu of primary tumor tissue from lung cancer patients. Mitochondrial mutations were identified in tumor samples and subsets of the identical mutations were also detected in the corresponding bodily fluid. Overall, eight out of nine matched serum DNA samples from cancer cases and nine out of nine sputum DNA samples contained an identical mutation to that detected in the primary tumor. It was found that mutations throughout the coding regions are frequently found in bodily fluids of cancer patients. These findings support the expectation that a relatively simple diagnostic test using the Mito Chip could provide early detection of lung cancer.

Smoking is the Prevailing Risk Factor for Lung Cancer

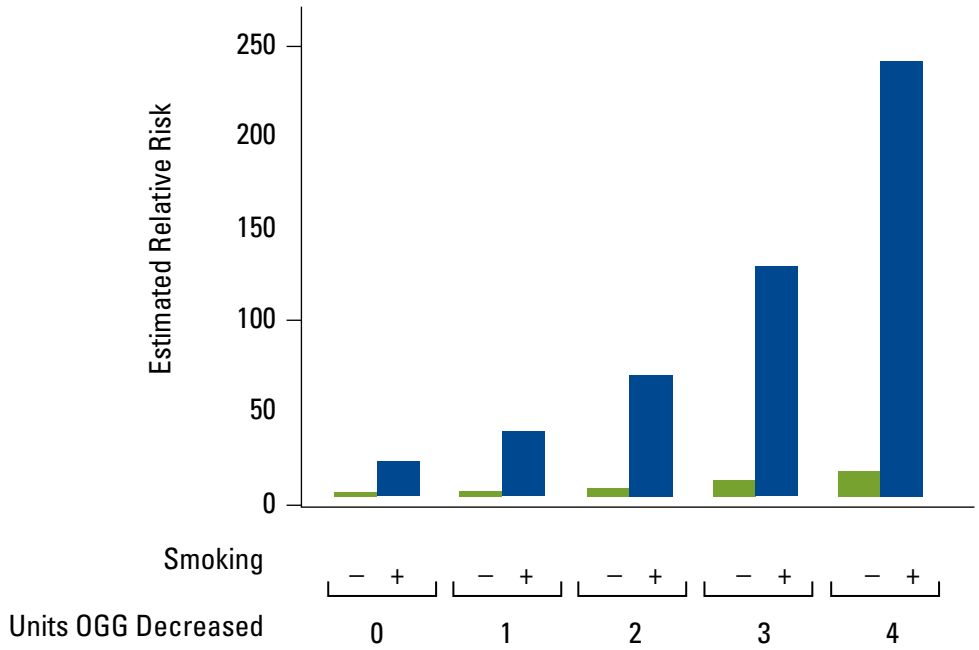
Over 80% of all lung cancers cases are attributed to smoking. People who have smoked for more than 10 years are at 12 to 50 times greater risk of developing lung cancer by age 75 than nonsmokers. Greater risk correlates with greater smoking history. EDRN investigators at the Johns Hopkins University and Lovelace have conducted numerous studies in heavy smokers as a high-risk group to discover lung cancer early detection biomarkers. Extensive gene methylation studies have revealed that current smokers with over 30 years of smoking history exhibit methylation profiles that overlap significantly with those from lung cancer patients with similar smoking history. These profiles are very different from nonsmokers. Similar observations were made by EDRN scientists at the University of Colorado examining chromosomal aberrations from bronchial airway epithelium. Persistent smoking induces considerable molecular changes in the lungs and airways that set the stage for disease progression to occur. Many of the expected biomarkers for early stage lung cancer progression are already present in current smokers, limiting their usefulness in determining lung cancer risk. These studies are now being extended to former smokers to determine if methylation profiles can distinguish them from former smoker lung cancer patients. It may then be possible to use methylation markers for early detection in former smokers.

DNA repair plays a major role in all cancer pathogenesis and lung cancer in particular, primarily because of its importance in removing DNA damage and preventing mutations. An EDRN group at the Weizmann Institute of Science is exploiting functional DNA repair enzyme assays as biomarkers for risk assessment and early detection of lung cancer. They have previously shown that reduced activity of a specific DNA repair enzyme termed OGG (8-oxoguanine DNA glycosylase), which removes the oxidative defect 8-oxoguanine from DNA, is a risk factor for non-small cell lung cancer. Moreover, they found that the combination of low OGG activity and smoking causes a much greater estimated relative risk for lung cancer (see Figure 4-2) suggesting that OGG activity may be a useful biomarker of risk. The group is now expanding the repertoire of enzymatic DNA repair biomarkers by developing enzymatic assays for two additional DNA repair enzymes, AP (apurinic/apyrimidinic) endonuclease and methylpurine DNA glycosylase, that likewise repair oxidative DNA damage. In addition, researchers at Lovelace are investigating whether the identification of specific **haplotypes** of genes affecting DNA repair capacity can provide a more comprehensive profile of risk prediction and identify individuals that could benefit from chemoprevention.

Figure 4-2. Reduced DNA Repair as a Risk Biomarker for Lung and Head and Neck Cancers

EDRN investigators at the Weizmann Institute of Science have shown that low DNA repair activity (OGG) in combination with smoking is associated with a much higher risk of lung and head and neck cancers. DNA repair is a housekeeping process responsible for DNA maintenance and prevention of mutations. DNA damage is caused by byproducts of intracellular metabolism, as well as external chemicals and radiation, such as tobacco smoke and sunlight. DNA repair enzymes scan the DNA, identify lesions and repair them using several mechanistic strategies thereby minimizing genetic damage that can lead to cancer. Based on these results, screening of smokers for low DNA repair activity may be used to identify individuals at extra-high risk for lung cancer. These individuals may be more motivated to enter smoking cessation programs, thereby reducing their risk for developing the disease. In addition, smokers with low activities may be a high-risk and cost-effective cohort for lung cancer early detection methods, which are too expensive for general population screening.

Smoking and Low DNA Repair Activity Greatly Increases Lung Cancer Risk



Source: EDRN investigators at the Weizmann Institute of Science

Interesting corollary studies are being conducted at New York University looking into the nature of mutations induced by smoking that occur in critical tumor suppressor genes. One of these projects examined acrolein, a by-product of incomplete combustion of fossil oil and organic substances that is one of the most reactive and environmentally abundant aldehydes. Although acrolein content in cigarette smoke is 1,000-fold higher than polycyclic aromatic hydrocarbons, its role in cigarette smoke-related lung cancer remains unclear. Through its formation of DNA adducts, acrolein can cause mutations. The tumor suppressor gene *p53* is one of the most frequently mutated genes in human cancer and over 50% of smoking-related lung cancers have *p53* mutations. New York University researchers have found that the DNA adducts induced by cigarette smoke carcinogens such as acrolein and polycyclic aromatic hydrocarbons preferentially occur at *p53* mutational hotspots that are poorly repaired. Their results suggest that acrolein is a major etiological agent for smoking-related lung cancer and that it contributes to lung carcinogenesis through two detrimental mechanisms: DNA damage and inhibition of DNA repair.

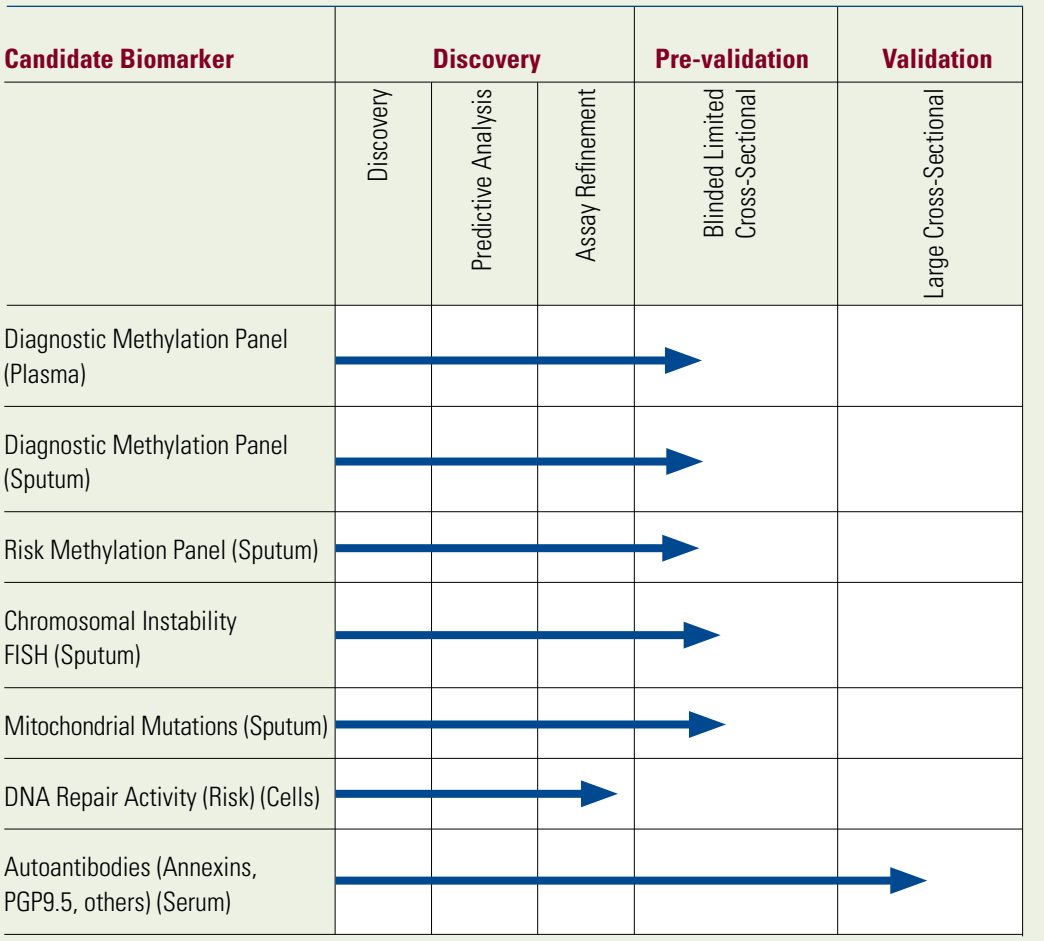
Researchers at the Fred Hutchinson Cancer Research Center (FHCRC) are working to develop blood tests for early detection of lung cancer. There is substantial evidence that the immune system reacts to the presence of tumors by inducing an antibody response to tumor antigens in a manner similar to the response that occurs against certain viruses such as HIV. This response may not be effective against the tumor but could be taken advantage of to detect the presence of the tumor or to develop more effective therapies directed against the tumor antigens. The identification of panels of such tumor antigens has utility in cancer screening and diagnosis in the same way seropositivity to HIV virus is used to identify individuals with HIV. Several approaches are currently available for the identification of tumor antigens. The FHCRC laboratory has pioneered a proteomic-based approach to identify those proteins, among thousands of proteins produced by tumors, that are most informative for cancer diagnosis based on their immunogenicity and specificity.

Ongoing work in the FHCRC laboratory is leading to discovery of more tumor antigens in addition to validation of previously identified ones, annexins and PGP 9.5. Results from ongoing validation studies are quite promising based on the blinded analysis of 60 sera collected as part of a previous study, the Beta-Carotene and Retinol Efficacy Trial (CARET). Sera were drawn from 30 subjects approximately a year before diagnoses of lung cancer and 30 sera were from matched controls. A significant difference in reactivity is observed for the designated antigens between those subjects that later developed lung cancer and the control subjects that did not.

Mesothelioma

Individuals exposed to asbestos have an increased risk of developing lung cancer and mesothelioma, a malignancy of the lung lining. There is an increasing realization by the scientific community that this population represents an ideal group to undertake early cancer detection because: (1) the probability that a disease marker will be significant is higher in this cohort than in the general, unexposed population; (2) asbestos-exposed cohorts can be closely followed in validation studies; (3) at-risk individuals are motivated to participate in follow-up studies; and (4) they present an opportunity to test early therapeutic intervention in patients with mesothelioma.

Candidate Lung Cancer Biomarkers



Two markers, serum mesothelin-related peptide (SMRP) and osteopontin, are the subject of EDRN investigations. Mesothelin is a protein attached to the cell surface of mesotheliomas, ovarian cancers and pancreatic cancers, that is thought to have a role in cell adhesion and cell-cell communication. One member of the mesothelin family is SMRP. Recently, an EDRN investigator in Australia reported determination of SMRP in serum with a sensitivity of 83% and specificity of 95% in the first 48 malignant mesothelioma patients tested. Changes in serum SMRP levels parallel clinical course/tumor size and SMRP was elevated in 75% of a larger cohort of patients at diagnosis.

The EDRN laboratory at New York University BDL found that another protein, osteopontin, could be an early marker for mesothelioma. Osteopontin is overexpressed in many cancers, including lung, where it mediates cell-matrix interactions and is regulated by proteins in cell-signaling pathways that have been associated with asbestos-associated cancer. In a study that compared serum osteopontin levels of patients with mesothelioma to individuals exposed to asbestos but without cancer, serum osteopontin levels rose with duration of asbestos exposure (0-9 years versus 10+ years) and degree of changes on an x-ray or CT scan (plaques and fibrosis versus other lesser findings). The mean serum osteopontin

Candidate Mesothelioma Biomarkers

Candidate Biomarker	Discovery			Pre-validation	Validation
	Discovery	Predictive Analysis	Assay Refinement	Blinded Limited Cross-Sectional	Large Cross-Sectional
SMRP and Osteopontin (Serum/Plasma)	→	→	→	→	
MMP9 (Serum/Plasma)	→	→			

level in individuals with mesothelioma was significantly higher than in the group exposed to asbestos, 77.6% sensitivity and 85.5% specificity when comparing the group exposed to asbestos to the group with mesothelioma. This study was the first to recognize that serum osteopontin levels could possibly distinguish persons with asbestos exposure without cancer from those with exposure who have developed pleural mesothelioma.

Definition of malignant mesothelioma:
A rare type of cancer in which malignant cells are found in the sac lining the chest or abdomen. Exposure to airborne asbestos particles increases one's risk of developing malignant mesothelioma.

U.S. and Australian investigators are now designing trials to validate whether these two markers and others could be used to screen for mesothelioma. A validation study of SMRP and osteopontin is under way. (See Chapter 6, Validation Studies, Case 4.) This study involves many noted cohorts including: the Selikoff Foundation at Mt. Sinai in New York; the Prostate Lung Colon and Ovarian Cancer Screening Trial; the CARET chemoprevention trial; the Center for Asbestos Related Diseases in Libby Montana where vermiculite was contaminated with tremolite asbestos; and Cappadocia Turkey where mesothelioma is epidemic due the presence of mineral fibers in building materials extracted from the neighboring mountains. Specimens are actively being collected from these sites where, hopefully, a group of markers will predict whether mesothelioma is developing in high-risk individuals exposed to asbestos. ■

CHAPTER FIVE

Prostate and Other Urological Cancers

"The EDRN has had an immense impact on discovery by providing samples, infrastructure and a multi-disciplinary team to make these discoveries happen. The EDRN will be very integral in the validation of these gene-fusion based biomarkers by providing a framework for systematic biomarker validation. It was really the only avenue for focused systematic biomarker discovery and validation."

Arul Chinaiyan, M.D., Ph.D.
Principal Investigator
EDRN Biomarker Development Laboratory
University of Michigan

PROSTATE CANCER is the most frequently diagnosed non-skin cancer in men in the United States. The prevalence of the diagnosis makes the disease a major health burden. While many men will die from prostate cancer, a majority of them will survive the disease as it is not uniformly fatal. Identification of aggressive forms of the disease is needed to spare men who might not need extensive treatments.

Bladder cancer is less prevalent than prostate cancer, yet four times more common in men than women and also twice as common in white men than black men. A priority for early detection of this disease is to identify cancers when they are superficial (early stage); however, even superficial bladder cancers are varied in their genetic makeup.

Kidney cancers can be successfully treated when diagnosed at an early stage. Renal cell carcinoma (RCC) is the most lethal of the common urologic cancers, with approximately 40% of patients eventually dying of disease.

The major focus of the EDRN Prostate and Urologic Cancer Collaboration Group is towards discovery and **validation** of **biomarkers** for early detection and risk assessment of urological cancers, including prostate, bladder and kidney.

In the last 2 years, the group developed standard reference materials, primarily plasma and serum (cases and matched controls) for detection and evaluation of prostate cancer biomarkers. Urine reference sets are being developed for bladder and prostate cancers. These reference sets are being used to commence blinded prevalidation and validation studies of candidate biomarkers. Two multi-institutional validation studies and five prevalidation studies have also begun in these disease areas.

EDRN Prostate and Urologic Cancers Collaborative Group Members

Daniel W. Chan, Ph.D., Chair
Johns Hopkins Medical Institute

O. John Semmes, Ph.D., Co-Chair
Eastern Virginia Medical School

Joseph Bigley, M.Sc.
OncoMethylome Sciences, Inc.

Paul Cairns, Ph.D.
Fox Chase Cancer Center

David Chia, Ph.D.
University of California, Los Angeles

Arul M. Chinnaiyan, M.D., Ph.D.
University of Michigan

Bogdan Czerniak, M.D., Ph.D.
University of Texas M. D. Anderson Cancer Center

Elefterios Diamandis, Ph.D.
Mount Sinai Hospital, Toronto

Sandra M. Gaston, Ph.D.
Beth Israel Deaconess Medical Center

William E. Grizzle, M.D., Ph.D.
University of Alabama at Birmingham

Jacob Kagan, M.S., Ph.D.
National Cancer Institute

Robin J. Leach, Ph.D.
University of Texas at San Antonio

Daniel W. Lin, M.D.
University of Washington

Alvin Y. Liu, Ph.D.
University of Washington

Alan W. Partin, M.D., Ph.D.
Johns Hopkins University

Martin Sanda, M.D.
Beth Israel Deaconess Medical Center

Mark P. Schoenberg, M.D.
Johns Hopkins University

Lori Sokoll, Ph.D.
Johns Hopkins Medical Institutions

Sanford Stass, M.D.
University of Maryland School of Medicine

Ian M. Thompson, M.D.
University of Texas at San Antonio

Dean Troyer, M.D.
University of Texas at San Antonio

Robert W. Veltri, Ph.D.
Johns Hopkins University

Prostate Cancer

Prostate cancer accounts for about one-third of all cancers found in men in the United States and is the second most common cause of cancer-related deaths. Recent advances indicate that prostate cancer is caused by both genetic and epigenetic alterations.

Definition of prostate cancer:
Cancer that forms in tissues of the prostate (a gland in the male reproductive system found below the bladder and in front of the rectum). Prostate cancer usually occurs in older men.

Estimated new cases and deaths from prostate cancer in the United States in 2007:
New cases: 218,890
Deaths: 27,050

Risk Factors

The search for the causes and ultimate prevention of prostate cancer entered a new era with recent developments allowing the correlation of environmental exposures with genetic/epigenetic variation and patient outcomes. Investigators at the University of Texas San Antonio have developed a prostate cancer risk-calculator that is based on factors such as PSA levels, digital rectal exam results (DRE), race, age and family history. Confirmation of the utility of this tool was demonstrated in a recently published study by the EDRN CEVC San Antonio Center for Biomarkers of Risk of Prostate Cancer (SABOR). This tool synchronizes currently established risk factors into a single composite score that can be used to identify those at high-risk for inclusion in prospective biomarker studies. Future refinements may include adding elements such as detrimental and protective polymorphisms in genes associated with critical pathways (for example, enzymes involved with androgen metabolism, DNA repair and hereditary susceptibility genes). The calculator can be accessed at http://www.compass.fhcrc.org/edrnnci/bin/calculator/main.asp

Genetic and Epigenetic Alterations as Cancer Biomarkers

The University of Michigan Biomarker Developmental Laboratory discovered frequent chromosomal rearrangements that juxtapose the *TMPRSS2* **promoter** and first non-coding exon to genes of the *ETS* family of oncogenes (*ETV1, ETV4* and *ERG*). **Fluorescence *in situ* hybridization (FISH)** analysis revealed that the majority of prostate cancers (~60%) harbor these rearrangements. This is the first demonstration of chromosomal rearrangements in epithelial cancer. These chromosomal rearrangements offer distinct advantages over the current biomarkers for prostate cancer, such as PSA, because they occur only in the cancerous cells and are partly responsible for the transformation mechanism. Current research is examing whether these rearrangements can be detected in urine sediment.

PCA3 is a non-coding prostate-specific mRNA that was reported to be frequently over-expressed in prostate tumor cells. Investigators at Johns Hopkins University are collaborating with Gen-Pobe, Inc. and Diagnocure, Inc. in the analysis of *PCA3*. A prototype **quantitative** *PCA3* urine test demonstrated potential as an adjunct to current methods for prostate cancer diagnosis and additional studies are in progress.

Epigenetic modification of DNA (particularly hypermethylation of CpG islands 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 mechanism is an alternative pathway for inactivation of tumor suppressor genes such as *p16* and *APC* in a variety of cancers. An EDRN laboratory at Johns Hopkins University reported that promoter **methylation** of several genes is a common feature of prostate cancer and high-grade prostatic intraepithelial neoplasia (HGPIN), the noncancerous growth of cells lining the internal and external surfaces of the prostate gland which may increase the risk of developing prostate cancer. The team assembled a panel of methylated genes as a new molecular marker for early cancer detection. The assay is based on the percentage of methylated alleles (PMA). PMA values of *APC* and *RARß2* are higher in HGPIN, carcinoma and normal prostate tissue; however, the median PMA values for all three genes is higher in prostate cancer.

Clonal expansion in cells carrying methylated alleles (*APC, GSTP1* and *RARß2*) is observed in HGPIN and prostate carcinoma and is consistent with cancer progression. GSTP1 promoter methylation is mainly observed in prostate carcinoma and some HGPIN lesions, representing an important marker for the transition to invasive neoplasia. The laboratory also developed a non-invasive test for prostate cancer based on a quantitative methylation-specific **polymerase chain reaction** (QMSP) of multiple genes in urine sediment DNA. A combination of four genes (*p16, ARF, MGMT* and *GSTP1*) theoretically

permits the detection of 87% of prostate cancers with 100% **specificity**. A separate multi-center study led by other investigators at Johns Hopkins focuses on the *GSTP1* methylation assay.

Proteomic Alterations as Biomarkers

CD90 (Thy-1) is a cell surface protein frequently over-expressed in prostate cancer and T-cells. Investigators at the University of Washington recently discovered that increased levels of the protein *CD90* was present in all of the 30 tumors examined. The increased levels were detected only immediately around the tumor, in the stromal cells. In addition, increased expression of *CD90* peptide fragments have been detected in all urine samples of prostate cancer patients tested so far. Currently, the laboratory is developing an **enzyme-linked immunosorbent assay (ELISA)** for quantitative detection of *CD90* in urine.

CD10, an enkephalinase, is a 100kDa transmembrane **glycoprotein** involved in the cleavage and inactivation of peptide hormones important for signal transduction including the enkephalins, bombesin and substance P. The biological function of these potential *CD10* substrates in the prostate is unknown. However, *CD10* is strongly expressed by normal prostatic luminal epithelial cells. A high percentage of prostate tumors show an early loss of *CD10* expression. Research is planned to examine *CD10* expression in urine sediment.

JM27 is an androgen-regulated gene expressed in the prostate, testis and the uterus. This potential serum marker was originally identified by Matritech, Inc. EDRN investigators at Johns Hopkins University recognized that *JM27* protein is highly expressed in serum of patients with severe forms of benign prostatic hyperplasia (BPH). A serum-based ELISA was developed, tested and found to distinguish between symptomatic and asymptomatic men with BPH. The **sensitivity** and specificity of the assay are 90% and 77%, respectively. Interestingly, the presence of prostate cancer in these men does not appear to alter the marker levels. Doxazosin has been used to treat BPH down-regulated *JM27* protein expression. This is the first reported serum-based marker for severe BPH.

A Johns Hopkins EDRN laboratory in a collaboration between Beckman Coulter, Inc. performed a blinded test to evaluate the utility of a new isoform of PSA, %proPSA. The %proPSA alone and a model of PSA derivatives that included proPSA, had better overall clinical utility for prostate cancer detection in this blinded standard reference set than did free PSA, BPSA and testosterone. These findings provide the rationale for broader validation studies to determine whether %proPSA can supplant other multiple molecular PSA assays for improving accuracy of prostate cancer screening.

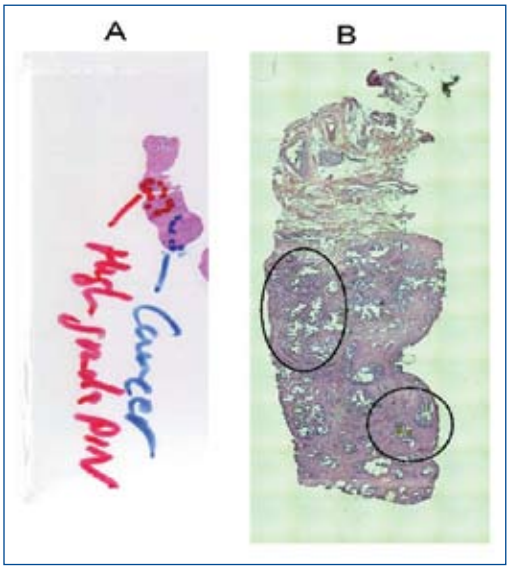
EDRN investigators completed validation studies of proteomic patterns as potential diagnostic markers for prostate cancer detection. They were able to confirm the portability and reproducibility of the test, but found that it did not perform well enough to advance to further testing. (See Chapter 6, Validation Studies, Case 5, for more information.)

Autoantibodies against peptides derived from prostate cancer tissue could be used as the basis for a screening test for prostate cancer. This suggestion is based on recent observations from laboratories at the University of Michigan and Beth Israel-Deaconess Hospital where patients with prostate cancer produced antibodies against N-methylacyl-coenzyme A racemase (AMACR), which is frequently overexpressed in prostate cancer. This autoantibody had 72% specificity and 62% sensitivity in detecting prostate cancer. Following on this observation, the laboratory built a phage display prostate cancer peptide library to screen for additional autoantibodies in prostate cancer patients. The laboratory developed a panel of 22 autoantibodies that displayed an 88% specificity and 82% sensitivity in discriminating between prostate cancer patients and the control group. This panel of peptides outperformed PSA in distinguishing between the two.

The ability to localize and follow changes at the molecular level by imaging the protein distributions in specific tissues is a promising improvement in pathological examination of specimens and as a discovery tool of markers for early detection. Investigators at the Eastern Virginia Medical School are applying **Matrix-Assisted Laser Desorption/Ionization Mass Spectrometric Imaging (MALDI-MSI)** for spatial visualization of peptides and proteins specific to different cell types to delineate differentially expressed proteins in various pathological lesions of prostate cancer (e.g., evaluate peptide expression pattern differences between HGPIN and prostate cancer). If successful, this technology could be applied to better classification, grading and staging.

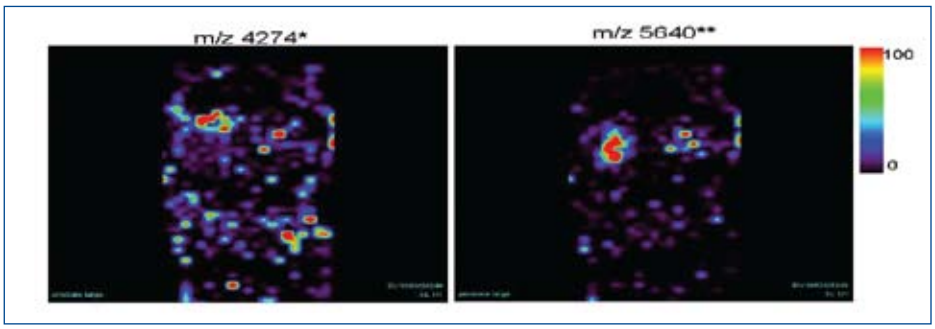
As shown in Figure 5-1, a prostate tissue section MALDI-MSI was used to analyze the contained regions of HGPIN and prostate cancer. Peaks found to be differentially expressed using MALDI-MSI can be seen in Figure 5-2.

Figure 5-1. Prostate tissue used for MALDI-MSI analysis



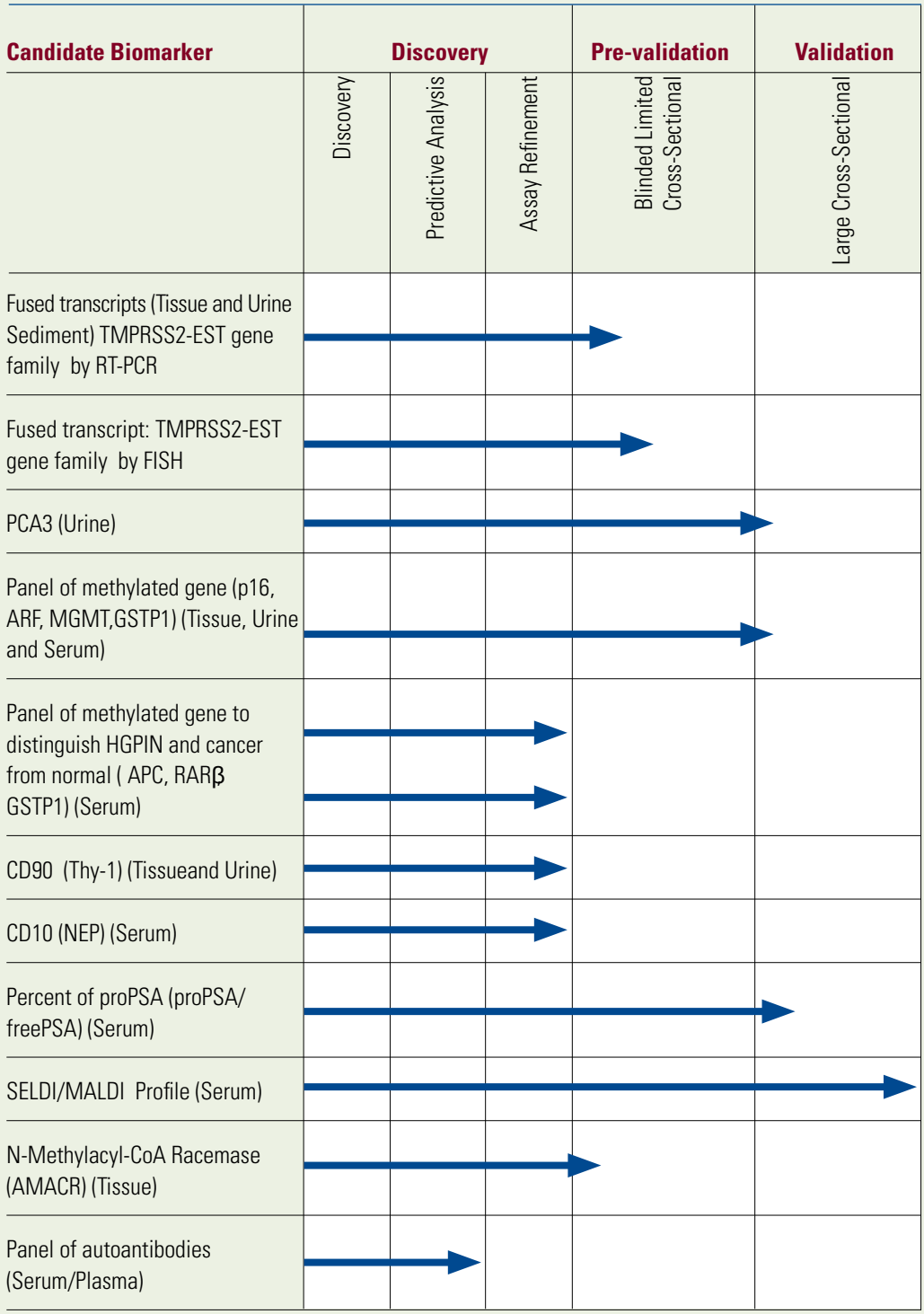
Source: EDRN investigators at Eastern Virginia Medical School

Figure 5-2. MALDI-MSI analysis of two peaks in frozen prostate tissue found upregulated in different microdissected cell types.



Source: EDRN investigators at Eastern Virginia Medical School

Candidate Prostate Biomarkers



Bladder Cancer

Over 90% of bladder cancer cases are transitional cell (urothelial) carcinoma (TCC), approximately 5% are squamous cell carcinoma and less than 2% are adenocarcinoma. Death due to bladder cancer is most often the result of muscle-invasive disease that accounts for approximately one-third of diagnoses. Superficial bladder tumors (non-muscle-invasive cancers) are a heterogeneous group of malignancies, including: papillary cancers that are limited to the mucosa; high-grade, flat and restricted to the epithelium; and invasive cancers that invade the sub-mucosa (lamina propria).

Definition of bladder cancer:
Cancer that forms in tissues of the bladder (the organ that stores urine). Most bladder cancers are transitional cell carcinomas (cancer that begins in cells that normally make up the inner lining of the bladder). Other types include squamous cell carcinoma (cancer that begins in thin, flat cells) and adenocarcinoma (cancer that begins in cells that make and release mucus and other fluids). The cells that form squamous cell carcinoma and adenocarcinoma develop in the inner lining of the bladder as a result of chronic irritation and inflammation.

Estimated new cases and deaths from bladder cancer in the United States in 2007:
New cases: 67,160
Deaths: 13,750

Aurora A (also known as *STK15* and *BTAK*) is a gene encoding a centrosome-associated serine/threonine kinase, which is frequently amplified and overexpressed in multiple human tumor cell types including bladder cancer. Overexpression of this gene is involved in tumorigenic transformation, induction of centrosome duplication-distribution abnormalities and **aneuploidy**. Recently, EDRN investigators at the University of Texas M. D. Anderson Cancer Center developed a FISH-based assay for the analysis of cells from urine to detect increased copy numbers of *Aurora A*. Three to four copies were detected in all bladder cancer specimens when the normal number should be two. Interestingly, patients with low-grade TCC had three to four copies of Aurora A, while patients with high-grade TCC had more than four copies of *Aurora A*. Normal copy numbers of *Aurora A* were detected in urine sediments of 17 unaffected controls. These studies suggest that amplification and overexpression of *Aurora A* is ubiquitous in bladder cancer and can be detected in bladder cells in urine.

At Johns Hopkins University, EDRN investigators identified somatic mitochondrial DNA (mtDNA) mutations in a variety of cancers. The frequency of mitochondrial mutations in these studies is high, with one-half to two-thirds of cancers harboring at least one mutation. These observations were independently verified, using the same samples by the team from the National Institute of Standards and Technology (NIST). Identical mtDNA mutations were detected in primary cancers and in urine sediments from the same of bladder cancer patients. There are several advantages in using mtDNA as a potential cancer biomarker including: (1) detection can be performed in noninvasive clinical samples such as from exfoliated cells in urine; (2) there are multiple copies of mtDNA in each mitochondrion; (3) there is an abundance of mitochondria (each cell contains hundreds to thousands of these organelles); and (4) most of the mutations and deletions are detected in limited regions.

EDRN investigators at Johns Hopkins also have established a panel of four methylated genes in bladder cancer (*CDKN2A, p14ARF, MGMT* and *GSTP1*) that displayed 100% specificity when evaluating paired DNA from samples, from primary tumor and from urine sediment. The paired samples displayed identical promoter methylation patterns. Of the 175 bladder cancer patients, 121 displayed promoter methylation in at least one of these genes, whereas all control subjects were negative for such methylation. Testing a small panel of genes by methylation-specific qPCR (qMSP) in urine sediment DNA is a powerful noninvasive approach for the detection of bladder cancer. Another panel (*APC, RASSF1A* and *p14ARF*) was independently established by EDRN investigators at the Fox Chase Cancer Center. A third panel (*CDH1, RASSF1A, APC* and *CDH13*) was established by investigators at the University of Texas Southwest Medical Center and the University of Texas M. D. Anderson Cancer Center. Larger independent confirmatory cohorts with longitudinal follow-up will be required in future studies to define the impact of these biomarkers on early detection, prognosis and disease monitoring before clinical application. A validation study of microsatellite analysis on urine is also under way. (See Chapter 6, Valiation Studies, Case 2.)

Candidate Bladder Cancer Biomarkers

Candidate Biomarker	Discovery			Pre-validation	Validation
	Discovery	Predictive Analysis	Assay Refinement	Blinded Limited Cross-Sectional	Large Cross-Sectional
Aurora A (Tissue and Urine)	→	→	→		
Aurora B and C (Tissue and Urine)	→				
Alterations in Mitochondrial DNA (Urine)	→	→	→	→	
Panel of Methylated DNA sequences (CDKN2A, ARF, MGMT, GSTP1) (Tissue and Urine)	→	→	→		
Panel of Methylated DNA sequences (APC, RASF1A, p14) (Urine and Tissue)	→	→	→		
Panel of Methylated DNA sequences (CDH1, RASF1A, APC, CDH13) (Urine and Tissue)	→	→	→		
Microsatellite Analysis (MSA) (Urine)	→	→	→	→	→

"The EDRN has truly reinvented scientific investigation in the United States. Traditionally, we have been a ruggedly individualistic scientific culture in which the individual's achievements are paramount. Unfortunately, when we face the challenge of early detection of cancer and the discovery and validation of biomarkers for this lethal group of diseases, one individual or one institution alone simply cannot achieve that goal. It has been a very satisfying experience to watch this incredibly dedicated group of individuals who have subordinated their personal rewards to those of the group and, in so doing, created the foundation for the early diagnosis and cure of cancer."

Ian M. Thompson, M.D.
Principal Investigator, EDRN Clinical Epidemiology and Validation Center
University of Texas Health Science Center

Renal/Kidney Cancer

In 2007, a projected 51,190 patients will be newly diagnosed with kidney and renal pelvis cancers and an estimated 12,890 will die from the disease. Although surgical treatment is efficient for localized cancer, 20% to 30% of patients with localized disease at presentation and 25% of patients with locally advanced or metastatic disease will develop systemic recurrence. Early detection of kidney cancer is essential for successful treatment.

Recently, EDRN investigators at Fox Chase Cancer Center developed a panel of six tumor suppressor genes *VHL, p16, p14, APC, RASSF1A* and *TIMP-3* that are frequently methylated in kidney cancer but not in normal kidney. An identical pattern of hypermethylation to that found in the tumor could be detected in the corresponding pre-operative urine DNA with high sensitivity and specificity (normal controls were methylation negative). As was discussed for bladder cancer, development of qMSP assay of this specific panel of genes offers promise for early detection of renal cancer from urine samples. ■

Definition of kidney cancer:
Cancer that forms in tissues of the kidneys. Kidney cancer includes renal cell carcinoma (cancer that forms in the lining of very small tubes in the kidney that filter the blood and remove waste products) and renal pelvis carcinoma (cancer that forms in the center of the kidney where urine collects). It also includes Wilms' tumor, which is a type of kidney cancer that usually develops in children under the age of 5.

Estimated new cases and deaths from kidney (renal cell and renal pelvis) cancer in the United States in 2007:
New cases: 51,190
Deaths: 12,890

Candidate Kidney Cancer Biomarkers

Candidate Biomarker	Discovery			Pre-validation	Validation
	Discovery	Predictive Analysis	Assay Refinement	Blinded Limited Cross-Sectional	Large Cross-Sectional
Panel of Methylated DNA sequences (VHL, p16, p14, APC, RASSF1A and TIMP-3) (Urine)		→			

Part Two

Process and Collaboration

CHAPTER SIX

Validation Stages and Processes

"While discovering and validating a biomarker that is associated with cancer is relatively easy, discovering and validating a biomarker that has clinical utility is very challenging. The reason is that to show clinical utility, a new biomarker needs to show advantages over the current clinical diagnostic practice which has been evolved and optimized during decades of medical research. However, the potential payoff is huge. A single discovery and validation of a marker for a major cancer would justify all the resources invested in EDRN because of the direct clinical relevance."

Ziding Feng, Ph.D.
Principal Investigator
EDRN Data Management and Coordinating Center
Fred Hutchinson Cancer Research Center

THE EARLY DETECTION Research Network (EDRN) takes a systematic approach to **biomarker validation**. A five-phase methodology was established as both a standard and a roadmap for successfully translating research on biomarker applications from the laboratory to the bedside. Designed to expedite procedures to evaluate and validate biomarkers for clinical application during the early stages of investigation, these five phases, shown in Figure 6-1, are:

- Phase 1: Discovery through exploratory studies to identify potentially useful biomarkers.
- Phase 2: Validation via studies that determine the capacity of biomarkers to distinguish between people with cancer and those without cancer (**sensitivity** and **specificity**).
- Phase 3: Studies to assess the capacity of a biomarker to detect preclinical disease by testing the marker against tissues collected longitudinally from research cohorts.
- Phase 4: Prospective screening studies.
- Phase 5: Large-scale population studies to determine overall impact of screening on health outcomes in the target population.

Within the Network structure, the Biomarker Developmental Laboratories (BDLs) develop and characterize new biomarkers, or refine existing biomarkers (Phase 1 and Phase 2). The Biomarker Reference Laboratories (BRLs) serve as the resource for clinical and analytical validation of biomarkers, including development of technology, standardization of assay methods and refinement of existing methods. The Clinical Epidemiology and Validation Centers (CEVCs) conduct or participate in early stages (Phase 2 and Phase 3) of clinical validation and epidemiological research for the application of biomarkers.

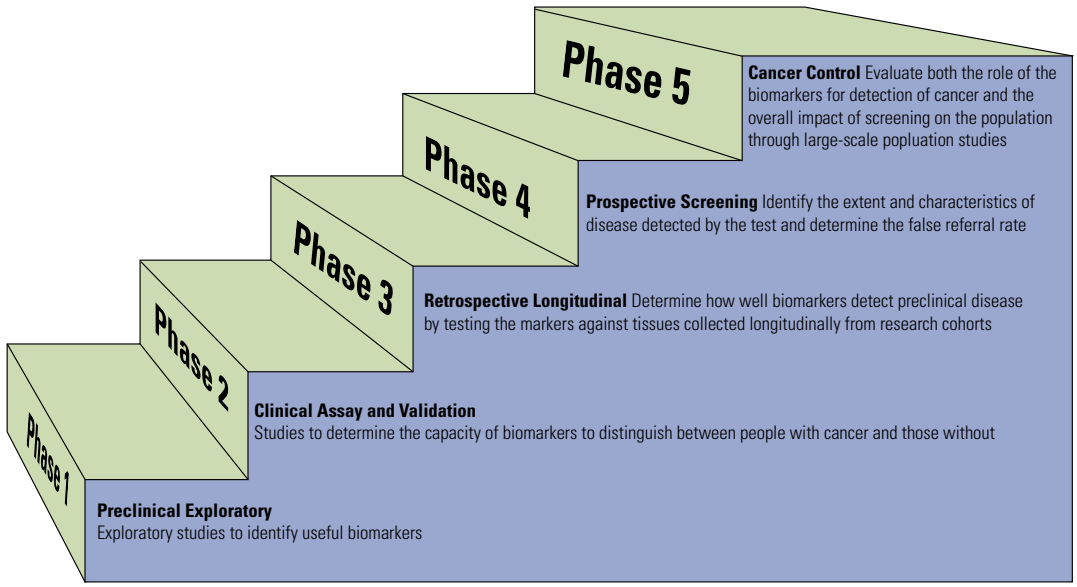
EDRN's ability to effectively organize the resources to conduct validation studies is highlighted below. In the clinical research community, such studies are generally rare since few BDLs ever achieve the necessary requirements or suitable resources.

Moving Discovery in Phases: Standard Specimen Reference Set

A biomarker that looks very promising in its initial laboratory work may not hold up when it undergoes the rigorous validation process that EDRN performs. The Network is working to develop discovery methods to increase the likelihood of identifying those markers that perform well in both the discovery phase and in validation.

One challenge facing investigators in this process is gathering properly stored biological specimens needed to test biomarkers. Often, an investigator obtains specimens from colleagues. The sample source from individuals with cancer is different from the source of specimens from individuals without cancer. Testing markers on such convenience specimens runs the risk of finding markers that are not associated with the disease of interest, but which show the differences in how specimens were collected (e.g., study population, method of collection, storage conditions).

Figure 6-1. Five Phase Approach to Biomarker Translational Research



Source: *Journal of the National Cancer Institute* 93, 1054-1061, 2001

Reference Sets Enable Accelerated Validation

A common problem encountered in assessing biomarkers worthy of clinical validation is that biomarker developmental work typically has been performed on samples from cases and controls collected in a variety of ways. This makes comparisons of biomarkers from different laboratories difficult and subject to significant bias. With the creation of shared reference sets of specimens from well-characterized cancer cases and matched controls, EDRN will overcome many of the logistic and design issues in preliminary and advanced biomarker validation. Already these reference sets enable direct performance comparisons of biomarker panels from different laboratories. This resource is accessible to any investigator within or outside of EDRN based on a common and transparent set of criteria used to evaluate applications. Interested scientists can obtain further details and request forms on existing reference sets at the EDRN web site (http://edrn.nci.nih.gov/resources/sample-reference-sets).

EDRN's solution to these difficulties is to create **standard specimen reference sets (SSRSs)**—collections of high-quality, well-characterized specimens that can be used for discovery and early validation of potential markers. By taking advantage of its large, diverse group of CEVCs, EDRN is able to create SSRSs with controls well-matched to cases on risk factors, as well as specimen collection, processing and storage conditions. Careful design by DMCC statisticians, in collaboration with EDRN investigators, ensures that the SSRSs are sufficiently powered to detect clinically important markers. The SSRSs include many different types of control individuals—ones with no disease, ones with benign diseases, ones with other types of cancer—so that the specificity of markers against potentially confounding conditions can be evaluated. Different SSRSs are created for different screening scenarios. For example, EDRN, in collaboration with Specialized Programs of Research Excellence (SPORE) investigators, created one SSRS to test markers that would be applied as a screen to a population at high risk for lung cancer and is creating another SSRS to test markers in individuals with abnormalities found on computerized tomography (CT) screening of the lung. EDRN is creating both retrospective SSRSs from previous cohorts as well as prospectively collecting specimens for other SSRSs.

Request for Biomarkers

EDRN publicly solicits potential cancer biomarkers from the greater scientific community to facilitate translational validation. The Network has a history of allowing outside investigators to join with EDRN scientists and thus gain access to clinical samples, reference sets and laboratory resources necessary for validation studies. Financial support for such studies is also available pending approval via a review process involving the appropriate organ site Collaborative Group. A number of prevalidation studies listed in this chapter were initiated via this solicitation.

A typical patient specimen may be divided into 20 or more samples or aliquots. Thus, as different investigators evaluate their markers in the SSRS and the resulting data are centrally deposited, DMCC statisticians have the ability to examine panels of markers combining all of these data together. By combining information from markers of different types (e.g., proteins and DNA **methylation**), the centralized data allow the creation of panels of markers that cannot be done by the individual laboratories and that may provide a multi-factor combination of markers that is more sensitive and specific than any single marker.

By June 2007, EDRN had created SSRSs for prostate, ovarian and lung cancers. The prostate cancer SSRS was so popular that it is already exhausted and a second prostate cancer SSRS is being developed. Other SSRSs in development include those for breast, pancreatic, colon and bladder cancers. As reference sets are created, they are deposited at the Frederick, Maryland facility of NCI and advertised in appropriate journals. Application methods and the review process for each SSRS are clearly indicated. Details on all SSRSs are available on the EDRN Public Portal (http://www.cancer.gov/edrn).

Private Sector Licenses Encourage Partnerships with Public Sector

EDRN-supported research is attracting the private and public sectors into partnerships. More than 90 patents have been issued to individual investigators and more than 40 licenses granted to private sectors. Among recently arranged licenses are: Assays for GSTP-1 (prostate) to OncoMethylome Science; EPCA-1 and EPCA-2 (prostate) to Onconome; Gene Fusion Assay (TMPRSS2-ETS) to Gene-Probe; GP-73 (liver) to Beckman-Coulter and SMRP (serum mesothelin-related protein) and osteopontin to Fujirebio Diagnostics. These markers are being brought to EDRN for further validation studies.

Validation Studies

Case 1: Validation of Serum Markers for Early Detection of Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC), primary liver cancer, is the fifth most common tumor and the third cause of cancer-related deaths worldwide with a 5-year survival rate of less than 5%. The high mortality associated with HCC is primarily due to diagnosis at a late stage when the tumor is unresponsive to treatment. However, when diagnosed early, 5-year survival rates can be as high as 70%.

Alpha-fetoprotein (AFP) is the only serum marker currently available to detect HCC, but its specificity and sensitivity are low and early stage HCC often goes undetected. In a pilot study, an EDRN Associate Member showed that the level of des-gamma carboxyprothrombin (DCP) in sera was significantly better than AFP in differentiating patients with HCC from those with cirrhosis. Cirrhosis is the major risk factor, appearing in approximately 90% of patients with HCC.

EDRN sponsored a large multi-site trial to validate these observations and to determine if DCP can accurately detect early stage HCC in cirrhotic patients. Soon after commencement of the trial, AFP-L3%, a form of AFP that was recently approved by the Food and Drug Administration for risk assessment was added. DCP is measured using an **enzyme-linked immunosorbent assay (ELISA)** kit provided by Eisai Company; AFP and AFP-L3% are measured by a commercial test from Wako Diagnostics. This EDRN validation study has determined the sensitivity and specificity of DCP for the diagnosis of early HCC; performance characteristics of DCP, AFP and AFP-L3% singly and in combination; and whether demographic and etiology of underlying liver disease alter the expression of DCP, AFP or AFP-L3%. A publication of this data is pending.

Case 1: Serum Markers for Hepatocellular Carcinoma

Design:

- Multi-center case-control study
- 450 cases: modified TNM stage I and II HCC (eligible for liver transplant)
- 450 controls: cirrhosis without tumor
- All study data entered into the EDRN Validation Study Information Management System, a secure, web-based system at the Fred Hutchinson Cancer Research Center
- Sera, plasma and DNA from peripheral blood cells are collected for this trial and stored to validate new biomarkers as they are discovered

Data Quality Management Committee Chair:

Richard K. Sterling, M.D., Medical College of Virginia, Richmond, VA

Participating Institutions:

University of Michigan Medical Center, Ann Arbor, MI (PI: Jorge Marrero, M.D.)
Fred Hutchinson Cancer Research Center, Seattle WA
Mayo Clinic, Rochester, MN
Mayo Clinic, Jacksonville, FL
Mount Sinai Hospital, New York, NY
Stanford University, Palo Alto, CA
St. Louis University, St. Louis, MO
University of California, Los Angeles, CA
University of Pennsylvania, Philadelphia, PA

Milestones:

November 2004	Protocol completed
February 2005	Begin enrollment
July 2007	End enrollment
September 2007	Finish assays and begin data analysis
November 2007	Finish analysis

Status:

12/1/2007	Publication pending

Case 2: Bladder Cancer Detection by Microsatellite Analysis of Urinary Sediment, Multi-Institution Study

Bladder cancer is the fourth most common malignancy among American men and the seventh most common malignancy among American women. Seventy-five percent of these patients have superficial bladder cancers and 70% of patients with superficial disease relapse after initial treatment. Consequently, individuals with superficial bladder cancer require frequent surveillance for recurrence.

There is a need to improve the current practice of bladder cancer surveillance. Although urine cytology and cystoscopy are considered standard of care, they are less than optimal in detecting bladder cancer. The sensitivity and specificity of urinary cytology is less than 50%. Cystoscopy, an invasive procedure, has a sensitivity of 90-100%.

In a preclinical study, a panel of 15 microsatellite markers was shown to detect greater than 90% of bladder cancers, using DNA from cells in urine sediment. EDRN is

sponsoring a large multi-site trial to determine the usefulness of **microsatellite analysis (MSA)** in early detection and monitoring for recurrence of superficial bladder cancer. MSA is based on detection of genetic instability that results in gain or loss of heterozygosity.

This EDRN validation trial aims to determine:

(1) the sensitivity and specificity of MSA (a panel of 15 microsatellite markers) using urinary sediment to detect bladder cancer compared to cystoscopy and urine cytology;

(2) the temporal performance characteristics of MSA in urinary sediment; and

(3) which of the 15 individual markers or combinations of markers are most predictive of the presence of bladder cancer.

Case 2: MSA of Urinary Sediment for Bladder Cancer

Design:

- Case-control study
- 300 individuals with a superficial bladder urothelial malignancy, either incident or recurrent
- 100 healthy individuals with no known urologic disease
- 100 individuals with potentially confounding conditions (BPH, foreign bodies, hematuria, or GU infection)

Data Quality Management Committee Chair:

H. Barton Grossman, M.D., Department of Surgery, University of Texas M. D. Anderson Cancer Center

Participating Institutions:

School of Medicine, Baltimore, MD
(PI: Mark Schoenberg, M.D.)
Baylor College of Medicine, Houston, TX
Brigham and Women's Hospital, Boston, MA
CURC Carolina Urologic Research Center, Myrtle Beach, SC
Harborview Medical Center, Seattle, WA
LURN, Daytona Beach, FL
LURN, Orange City, FL
LURN, West Orange, NJ
University of Texas M. D. Anderson Cancer Center, Houston, TX
Memorial Sloan Kettering, New York, NY
Stanford University, Palo Alto, CA
University of Alabama, Birmingham, AL
University of Chicago, Chicago, IL
University of Michigan, Ann Arbor, MI
University of Rochester Medical Center, Rochester, NY
University of Texas, San Antonio TX
University of Toronto, Ontario, Canada
Washington University, St. Louis, MO

Milestones:

December 2003	Protocol approved
January 2004	First meeting of investigators
May 2006	Eleven urological clinics added to the study
September 2008	Final results expected

Status:

12/30/2007	282 cases and 210 controls enrolled

Case 3: Validation of Biomarker Consensus Panel for Early Detection of Ovarian Cancer, EDRN-SPORE-PLCO Phase II Study

Most cases of ovarian cancer are diagnosed at advanced stages, which is associated with poor survival. Yet when clinicians are able to make an early diagnosis, survival can reach up to 90%. While approaches to ovarian cancer screening might include pelvic examination and sonography, tests that can measure biomarkers in blood are likely to be the most cost-effective first-line screen.

CA-125, a serum marker currently used for ovarian cancer, gives a true positive result for about 50% of Stage I ovarian cancer patients, but is not an adequate early detection tool when used alone. Consequently, EDRN and NCI SPORE investigators have joined forces to develop a two-phase study to investigate a panel of biomarkers that could be used to screen for ovarian cancer.

In the first phase, which is nearing completion, investigators at Brigham and Women's Hospital, Fred Hutchinson Cancer Research Center, University of Texas M. D. Anderson Cancer Center and University of Pittsburgh Cancer Institute are validating biomarkers that performed well in preliminary studies in their respective laboratories. This is being done on a blinded test set of sera from 80 early-staged and 80 late-staged ovarian cancer cases, 160 controls with benign disease and 480 healthy controls. Results from these individual laboratories will be combined to form a biomarker consensus panel that most accurately detects early stage ovarian cancer.

In phase 2 of this study, this biomarker consensus panel will be used to test sera collected one or more years prior to diagnosis of ovarian cancer. These specimens and matched controls will come from NCI's Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO). The investigators' hypothesis is that a panel of biomarkers will have a sufficient lead time to identify ovarian cancer cases two or more years earlier than current tests allow.

Case 4: Biomarker Validation for Early Detection of Mesothelioma, American/ Australian Mesothelioma Consortium

Individuals exposed to asbestos are at high risk of developing mesothelioma, a malignancy of the lung lining. Mesothelioma is currently diagnosed by assessment of clinical and radiological findings and confirmed by tissue biopsy. Treatment options for mesothelioma have not proved successful and patients have a median survival time of 6-12 months.

Patients with early stage disease can survive five or more years if their tumor is promptly resected. Currently, less than 5% of mesothelioma patients are diagnosed at an early stage of disease. Thus, there is a need for a biomarker, or panel of biomarkers, that can predict the development of mesothelioma, or detect it at an early stage. Research by EDRN and other investigators strongly suggests that two serum proteins, serum mesothelin-related protein (SMRP) and osteopontin are biomarkers of early detection of mesothelioma and that these biomarkers might be useful to screen asbestos-exposed individuals.

EDRN initiated a validation trial to determine the performance of these two blood-based protein biomarkers in the diagnosis of early stage mesothelioma. The trial aims to determine:

(1) the performance of SMRP and osteopontin in case-control studies in retrospective specimens collected from asbestos-exposed individuals at a number of centers in the United States and Australia;
(2) the performance in a prospective study on longitudinal samples collected from several high-risk mesothelioma cohorts at several sites around the world; and
(3) the effectiveness of these biomarkers in detecting the onset of mesothelioma prior to diagnosis.

Case 3: Biomarker Consensus Panel for Ovarian Cancer

Design:

- Case-control study
- 80 early stage and 80 late-stage ovarian cancer cases
- 160 controls with benign disease
- 480 healthy controls

Participating Institutions:

Brigham and Women's Hospital, Boston, MA (Daniel Cramer, M.D., EDRN PI)
Fred Hutchinson Cancer Research Center, Seattle, WA (Nicole Urban, ScD., SPORE PI)
University of Texas M. D. Anderson Cancer Center, Houston, TX
Fox Chase Cancer Center, Philadelphia, PA
University of Pittsburgh, Pittsburgh, PA
University of Alabama at Birmingham, Birmingham, AL

Timeline for Completion of the Project:

March 2007	Phase I specimens sets were received by the assay sites (Boston, Houston, Pittsburgh, Seattle)
May 2007	Completion of assays on the Phase I specimens
Summer 2007	Completion of data analysis by the DMCC
October 2007	Final decision on the structure of the samples for the PLCO specimens and panel of ovarian cancer biomarkers
November 2007	PLCO specimens shipped to assay sites
December 2007	Assays on PLCO specimens completed and results to PLCO
January-March 2008	Discussion with PLCO regarding findings, forums for presentation and write-up

Milestones:

- Identification of a consensus panel comprising the biomarkers that is most informative when used singularly as well as in combination
- Assay preclinical sera from ovarian cancer patients enrolled in the PLCO trial

The New York University investigators and their Australian collaborators have involved many noted cohorts including those from: the Selikoff Foundation at Mt. Sinai in New York; the PLCO; the Beta-Carotene and Retinol Efficacy Trial (CARET) chemoprevention trial; the Center for Asbestos Related Diseases in Libby Montana; and from Cappadocia, Turkey. Specimens are actively being collected from these sites.

Case 5: SELDI-TOF-MS Serum Proteomic Profiling Does Not Reliably Detect Prostate Cancer

Initiated in 2003, the EDRN investigators started a multi-institutional collaborative project to validate **proteomics** patterns as potential diagnostic markers for cancer detection. This was triggered by the publication of a number of research articles in 2002-2003 on the use of protein expression patterns as potential biomarkers for ovarian, prostate, lung and other cancers.

Case 4: Biomarker Validation for Early Detection of Mesothelioma

Design:

- Case-control study
- 200 mesotheliomas
- 500 asbestos exposed individuals
- Most controls are asbestos-exposed individuals

Participating Institutions:

New York University, New York, NY
(PI, Harvey I. Pass, M.D.)
University of Western Australia, Perth, Australia
Peter MacCallum Cancer Institute,
Melbourne, Australia
University of Hawaii, Honolulu, HI
Fujirebio Diagnostics, Inc., Malvern, PA

Milestones:

Summer 2007	Protocol completed
Fall-Winter 2007-2008	Receipt of all samples

Status:

12/10/2007	All samples for validation have been blinded and are waiting analysis

EDRN investigators meticulously designed a three-stage protocol to validate the reproducibility of the platform (Stage 1), to validate diagnostic use of protein patterns (Stage 2) and to conduct clinical validation (Stage 3), using well-annotated, prospective specimens from stratified risk groups and prostate cancer cases and controls. Results from Stage 1 of the study confirmed the portability and reproducibility of the **surface-enhanced laser desorption-time of flight mass spectrometry (SELDI-TOF-MS)** platform. Encouraged by this finding, investigators conducted the second stage of the validation process.

The results from the Stage 2 study concluded that the results from previously published studies in which discrimination between prostate cancer and non-cancer was demonstrated is not generalizable. Earlier study samples likely had biases in sample selection that upon removal, as in the present study, resulted in inability of the technique to discriminate cancer from non-cancer cases. Thus, in the second phase of the planned validation process, the SELDI-TOF-MS-based protein expression profiling approach did not perform well enough to advance to the third (prospective study) stage. Results of the Stage 2 study, which are being submitted for publication, will discuss the impact these findings have on the biomarker discovery field and propose that the EDRN validation design be the standard protocol for analysis of biomarkers for disease detection.

Other Validation Studies

Early Detection of Prostate Cancer Based on Detection of PCA3 Transcript in Urine Supernatant

- PI: John Wei, University of Michigan, Ann Arbor, MI. Collaborators, Harry Rittenhouse, Alan Partin, Martin Sanda, Arul Chinnaiyan, Ziding Feng.
- Industrial Collaborator: Gen-Probe; PI: Harry Rittenhouse
- Status: protocol is being developed.

Case 5: Novel Protein Profiling Techniques for Prostate Cancer

Design:

- Case-control study

Participating Institutions:

Eastern Virginia Medical School, Norfolk, VA (PI, John O. Semmes, Ph.D.)
University of Alabama at Birmingham, Birmingham, AL
University of Texas Health Science Center, San Antonio, TX
University of Pittsburgh Cancer Institute Hillman Cancer Center, Pittsburgh, PA
Johns Hopkins University, Baltimore, MD
Center for Prostate Disease Research Walter Reed Army Medical Center, Washington, DC
Fred Hutchinson Cancer Research Center, Seattle, WA

Milestones:

February 2004	Stage I completed
September 2006	Stage II completed

Status:

January 2008	Reports in press (*Clinical Chemistry)*

Validation of Percent proPSA in Combination with PSA Isoforms for Early Detection of Prostate Cancer

- Industrial collaborator: Beckman-Coulter
- PI: Lori Sokoll, JHMI. Collaborators: Martin Sanda, Alan Partin, Ian Thompson, Dan Chan and Beckman-Coulter

Prevalidation Studies in the EDRN Pipeline

The feasibility and performance in relatively small case-control design studies for certain promising biomarkers in cancer diagnosis or risk prediction, termed prevalidation studies, are underway. The results will determine whether a larger cohort study should be considered. EDRN continues to receive proposals from investigators outside the Network who seek assistance in bringing their biomarkers forward for clinical validation. The following list describes a number of such ongoing studies that are likely to lead to full-scale validation studies.

Serum-Based Protein Markers for the Detection of Colorectal Cancer

- PIs: Robert Getzenberg, Johns Hopkins University and Robert Schoen, University of Pittsburgh
- Aim: 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.
- Projected clinical use: Screening for colorectal cancer.
- Biomarkers: Colon Cancer Specific Antigen-3 and -4, nuclear matrix proteins specific to colorectal cancer that can be measured in sera.

Early Detection of Bladder Cancer Using DNA Methylation Markers in Urine Sediment

- PI: Paul Cairns, FCCC
- Industrial Collaborator: OncoMethylome
- Status: The protocol for the study was completed and shared with the collaborators for comments (David Sidransky, Bogdan Czerniak, Adi Gazdar and Martin Sanda).

Early Detection of Prostate Cancer Based On Prostate Cancer Translocations in Urine Exfoliated Cells

- PI: Arul Chinnaiyan, University of Michigan
- Industrial Collaborator: Gen-Probe
- Status: Protocol is being developed.

Protein Markers of Lung Cancer

- PI: Samir Hanash, Fred Hutchinson Cancer Research Center
- Aim: To determine the efficacy of a panel of at least five biomarkers in blood for diagnosis of early stage lung cancer.
- Projected clinical use: Identify people in high-risk groups (smokers) with early stage lung cancer.
- Biomarkers: Autoantibodies to Annexins I and II and PGP9.5; C-reactive protein and serum amyloid A will also be analyzed to see if their inclusion in this panel can enhance the performance of the autoantibodies.

Barrett's Esophagus Progression Biomarkers

- EDRN PI: Stephen Meltzer, Johns Hopkins University
  SPORE PI: Richard Sampliner, University of Arizona
- Aim: To test a three-class stratification model for risk of progression from Barrett's esophagus to esophageal adenocarcinoma.
- Projected clinical use: Patients classified as 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.
- Biomarkers: Methylation status of three tumor suppressor genes (*p16, HPP1, RUNX3*) used in combination with four clinical parameters.

Ovarian Cancer Biomarker Validation Study

- PI (EDRN Associate Member): Gil Mor, Yale University (in partnership with LabCorp)
- Aim: To validate a panel of serum protein biomarkers for the detection of early ovarian cancers using multicenter patient specimen collections.
- Projected clinical use: Identify patients with early ovarian cancers that will enable further diagnostic evaluation and early clinical and therapeutic intervention.
- Biomarkers: Prolactin, osteopontin, leptin, insulin-like growth factor II (IGF-II), macrophage migration inhibitory Factor (MIF-2) and CA-125 will be analyzed by Luminex technology.

GSTP1 Methylation Marker for Prostate Cancer

- PI: Alan Partin, Johns Hopkins University
- Aims: To validate methylated GSTP1 and three additional markers as a panel for detection of prostate cancer in biopsy specimens.
- Projected clinical use: Diagnosis of patients with both positive digital rectal examinations (DRE) and "normal" PSA and in patients with rising PSA levels but negative biopsies.
- Biomarkers: *GSTP1, p16, ARAF, MGMT*.

Validation Study of Cervical Cancer Progression Biomarkers

- PI: Thomas Ried, NCI Center for Cancer Research
  EDRN PI: Elizabeth Unger, CDCP
- Aim: To validate biomarkers for progression to cervical cancer.
- Projected clinical use: Reduction in the need for repeated colposcopies and in the costs for screening cervical cancer.
- Biomarkers: Chromosomal gain of 3q and methylated genes used together in Pap smear samples.

Additional Studies Under Consideration

Mutations and Deletions in mtDNA as Markers for Bladder and Other Cancers

- PI: David Sidransky and Mark Schoenberg, Johns Hopkins Medical Institutes
- Industrial collaborator: None at this time
- Status: discussions.

Meythlated DNA Markers for Prostate Cancer

- PI: David Sidransky, Johns Hopkins Medical Institutes
- Industrial Collaborator: Oncomethylome
- Status: discussions. ■

CHAPTER SEVEN

Enabling Technologies

"Our investigations have shown that finding a suitable cancer biomarker (in blood) depends in part on the detection methodology used. In this regard, the EDRN network has funded different technologies, perhaps more powerful, to identify these markers. More importantly, the EDRN group has created a standardized panel of test material for validation. This cannot be done by individual research groups. Above all, the coordination among Marker Discovery, Marker Validation and Data Analysis made possible by EDRN is unique and crucial to the success of this program. The free exchange of data at regular intervals ensures that various expertise and pertinent experimental results are communicated to the community."

Alvin Liu, Ph.D.
Principal Investigator
EDRN Biomarker Development Laboratory
University of Washington

THE EARLY DETECTION Research Network (EDRN) is a leader in incorporating new technologies into research devoted to the discovery and development of **biomarkers**. Although EDRN investigators pioneer cutting-edge technologies, the Network invites collaboration from other scientists and companies to adapt their platforms to early detection diagnostic tests. This chapter highlights some of the novel technologies and supporting infrastructure implemented by EDRN to maximize progress through the use of well-designed interactive tools.

Nanotechnology Supports Sensitive Detection of Blood-Based Biomarkers

The detection of specific proteins in blood or other biological fluids is important for both clinical and research applications. Techniques for detection typically involve capturing the protein of interest from the blood using specific antibodies, then detecting this captured protein with an antibody linked to an amplification tag, such as a fluorochrome, enzyme or radioisotope marker. EDRN investigators are utilizing novel electronic device architecture for the detection of proteins in blood by taking advantage of a room-temperature carbon nanotube (CNT) network fabrication technology. The approach will form a charged circuit that is sensitive to changes in the amount of charge near the CNT network. Antibodies immobilized on the CNT surface serve to specifically bind proteins, thus altering the surface capacitance. As a model test system, investigators have demonstrated the quantitative detection and measurement of human prostate-specific antigen (PSA) added to calf serum.

xMAP Assay

Recently, LUMINEX Corporation introduced a novel protein array system (xMAP for Multianalyte Profiling) that allows for simultaneous quantitation of up to 100 soluble analytes in one sample. xMAP technology uses polystyrene microspheres internally dyed with differing ratios of two spectrally distinct fluorophores to create a family of 100 differentially spectrally addressed bead sets. Each of the 100 spectrally addressed bead sets can be conjugated with a capture antibody specific for a unique target protein. In a multiplexed assay, antibody-conjugated beads are allowed to react with sample (plasma, serum or cell culture supernatant). After washing, detection antibodies are added to a microtiter plate well to form a capture sandwich immunoassay. Using the xMAP assay (see Figure 7-1), thousands of beads can be analyzed in seconds, allowing up to 100 analytes to be measured in a 96-well microplate in one hour.

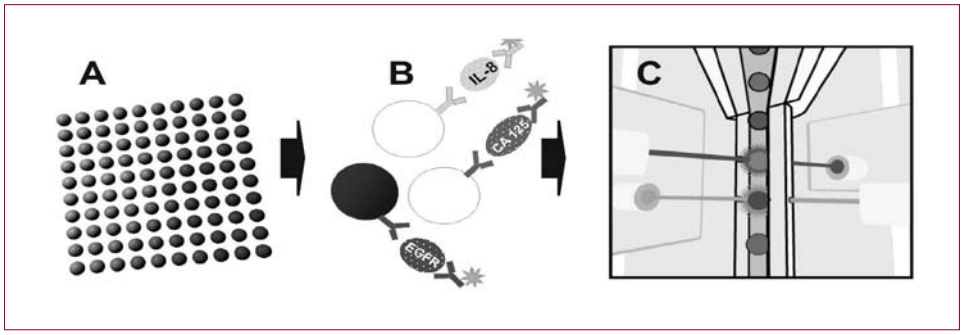
Investigators at the University of Pittsburgh are using the xMAP to test a number of biomarker panels based on cytokines, chemokines, angiogenic and growth factors for pancreatic, ovarian and lung cancers. Because xMAP permits simultaneous evaluation of many analytes, a large panel of analytes can be tested initially with this technology and then focused to an optimal panel for distinct cancers.

Multiple Platforms Accelerate Biomarker Discovery

Investigators at the University of Michigan integrated bioinformatics tools to mine gene expression data derived from prostate cancer cell lines, prostate tumors and other model systems. The team is a leader in the cataloging and bioinformatics analyses of DNA **microarray** data through the development of Oncomine (http://www.oncomine.org). They also developed a method termed Cancer Outlier Profile Analysis (COPA), which analyzes DNA microarray data for genes markedly over-expressed in a subset of cases.

The COPA transformation effectively compresses typical biomarker profiles characterized by general overexpression in cancer relative to normal tissue. At the same time, it accentuates the outlier profiles characterized by general low expression with marked overexpression in a fraction of cancer samples ultimately providing a list of outlier genes. This analysis successfully identified several well-known cancer genes in specific cancer types with well-documented recurrent chromosomal rearrangements or amplifications. The striking observation was made that two ETS transcription factors known to be involved in gene fusions in Ewing's sarcoma and myeloid leukemia, ERG (21q22.3) and ETV1 (7p21.2), were highly ranked outliers in multiple independent prostate cancer profiling studies and

Figure 7-1. xMAP Assay, a new protein array system, allows for simultaneous quantitation of up to 100 soluble analytes in one sample.



A. Analytes B. Antibody-Analyte Complex C. Detection System

Source: EDRN investigators at the University of Pittsburgh

furthermore, the outlier profiles of ERG and ETV1 were mutually exclusive. The discovery of ETS fusions with the prostate-specific gene *TMPRSS2* represents a paradigm shift for major epithelial tumors. The presence and high frequency of *TMPRSS2*:ETS fusions in prostate cancers suggest that this may be a causal event, similar to the role of recurrent rearrangements in hematological and mesenchymal malignancies.

Metabolomics in Breast Cancer

In collaboration with EDRN investigators at the University of California at San Francisco, scientists at Lawrence Livermore Laboratories developed a unique application of imaging mass spectrometry based on the **time-of-flight secondary ion mass spectrometer (ToF-SIMS).** This instrument is being used to achieve chemical mapping of breast cells and tissues. Investigators are using ToF-SIMS to cluster individual breast cancer cells from established cell lines into their respective groups, including discrimination of cell lines with distinct phenotypes. They also perform ToF-SIMS on paraffin-embedded formalin-fixed primary tumors as well as representative cell lines from which expression profiles have been previously obtained. Cluster analysis by ToF-SIMS is being correlated with transcriptomic/genomic profile analysis. Results are integrated into the genomic analysis that include total ion images, images based on masses of interest (ones identified by loading plots), images in red/green pseudocolor defining the differences and zoomed mass images of regions of interest, thus differentiating cancer cell lines from each other.

High-Throughput Sequencing to Detect Mitochondrial Mutations

Somatic mutations in the mitochondrial genome have recently been discovered to be characteristic of many cancers. To pursue mitochondrial DNA (mtDNA) mutations for cancer diagnostics, EDRN investigators at Johns Hopkins in collaboration with the Biomarker Reference Laboratory (BRL) at the National Institute of Standards and Technology (NIST) developed an oligonucleotide-based sequencing microarray called the MitoChip. This chip, now in its second version, enables resequencing of the entire mitochondrial genome of about 16,500 base pairs via a simplified and high-**throughput** process. Accuracy and reproducibility of sequences determined using automated software is very high and sufficient to sensitively identify mtDNA mutations. This technology is being applied to multiple cancers and preneoplastic lesions (lung, head and neck, bladder, Barretts esophagus, colorectal adenomas) to explore applications for early detection and diagnosis of cancer.

Sensitive High-Throughput ELISA Technology

The Breast and Gynecological Cancers collaborative group of EDRN is partnering with Meso Scale Diagnostics (MSD) to use their sensitive electrochemiluminescence-based technology to screen a large number of biomarkers for their potential to detect breast, endometrial and ovarian cancers. MSD will analyze EDRN serum reference sets for ovarian, endometrial and breast cancers. MSD **enzyme-linked immunosorbent assays (ELISA)** for serum-based measurements have sensitivities as low as 0.1 pg/ml, a dynamic range of 3-5 orders of magnitude, with rapid throughput. The **sensitivity** and mulitplexed formats of MSD assays enable testing of large numbers of biomarkers with small amounts of serum. These studies would form the basis for future studies using individual case specimens obtained through EDRN collaborators to identify early cancer detection markers, particularly for ovarian cancer for which early detection is currently very inefficient. This new technology assessment will be an important step towards development of suitable early cancer detection screening tests. MSD technology would be made accessible to EDRN investigators to incorporate into their study programs.

Nucleic Acid Programmable Protein Array

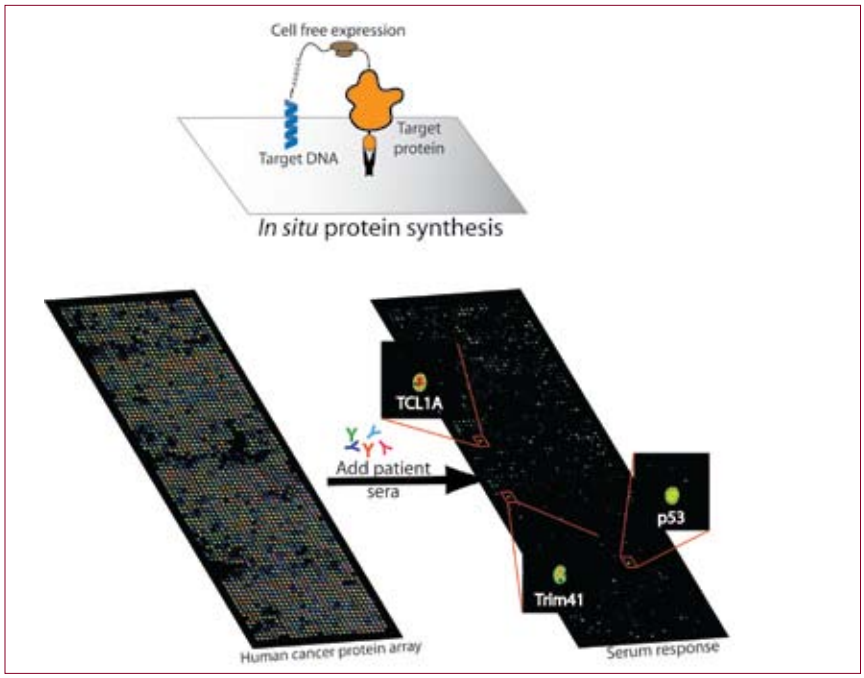
Most currently available methods for producing protein microarrays require purification of proteins for printing on the array. The laboratory at Harvard Medical School developed a novel DNA-based protein array technology called nucleic acid programmable protein array (NAPPA) where proteins are transcribed *in situ* from an immobilized DNA template using a cell-free expression system (see Figure 7-2). The freshly expressed proteins are captured via their epitope tag at the site of synthesis. This approach overcomes the need to separately express and purify proteins for printing. These microarrays are then used to identify autoantibodies directed to tumor antigens in cancer patient sera, with equivalent sensitivity and **specificity** to standard ELISA. Current EDRN developmental projects are adapting the NAPPA protein microarray technology for use in the rapid and efficient screening of sera from breast cancer patients for antibodies to 2,000 known and potential tumor antigens.

A number of promising activities complementing EDRN efforts in **genomics, proteomics** and **epigenomics** are being discussed with industrial partners who possess the resources and financial backing to initiate "incubator projects" with the Network. Technology developers will provide platforms, reagents and assays. In return, EDRN will provide specimens and expertise in conducting small projects on technology **validation**, refinement of assays and reagents and statistical interpretations for performance metrics. Some of the incubator programs under discussion concern transcriptomic analyses, gene **methylation** analyses and microRNA, comparative genomic hybridization and protein array analysis.

Figure 7-2. Nucleic Acid Programmable Protein Array Technology

This figure illustrates the DNA-based protein array NAPPA technology. Proteins are synthesized *in situ* from an immobilized DNA template using the cell-free expression system. The freshly expressed proteins are captured via their epitope tag at the site of synthesis. The approach overcomes the need to separately express and purify proteins for printing. The efficient expansion of over 2,000 full-length human proteins is confirmed using an anti-epitope tag (known as anti-glutathione-S-transferase) antibody.

Immunoprofiling of cancer patients using self assembling protein arrays



Source: *Current Opinion in Chemical Biology*, Joshua LaBaer and Niroshan Ramachandran, Vol 9, Author(s), "Protein microarrays as tools for functional proteomics," pp. 14–19, Copyright Elsevier (2005).

EDRN Knowledge Environment

EDRN's investment in informatics made it a leader in applying new technology for the NCI. Informatics plays a key role in supporting the scientific discovery process by building the infrastructure and tools that connect the EDRN research institutions together into a virtual knowledge system.

Coordinated discovery of biomarkers across cancer research centers provides an opportunity for the Network to increase the accuracy of study results. However, the distributed nature of EDRN represents a challenge for building a bioinformatics infrastructure to capture and distribute the science and ancillary data acquired during biomarker studies within the enterprise.

EDRN requires a knowledge system that links highly diverse systems together into a virtual data grid to support new analysis mechanisms ultimately identifying and validating new biomarkers. This data grid allows for linking loosely related data items across a highly heterogeneous, distributed environment.

The EDRN Knowledge System promises to dramatically improve the capability of scientific research by enabling real-time access to a variety of information that crosses institutional research center boundaries. While there are clear scenarios for how such a system can improve the discovery process, flexibility and agility are crucial to support new approaches to discovering cancer biomarkers. By decomposing the knowledge system into a set of communicating information services based on a domain information model, the Network is able to support the evolutionary needs of the program. Clearly, virtualized data grids are in their infancy, but the needs of programs like EDRN are demonstrating the benefits and the criticality for bringing scientific research endeavors together into a secure, integrated enterprise.

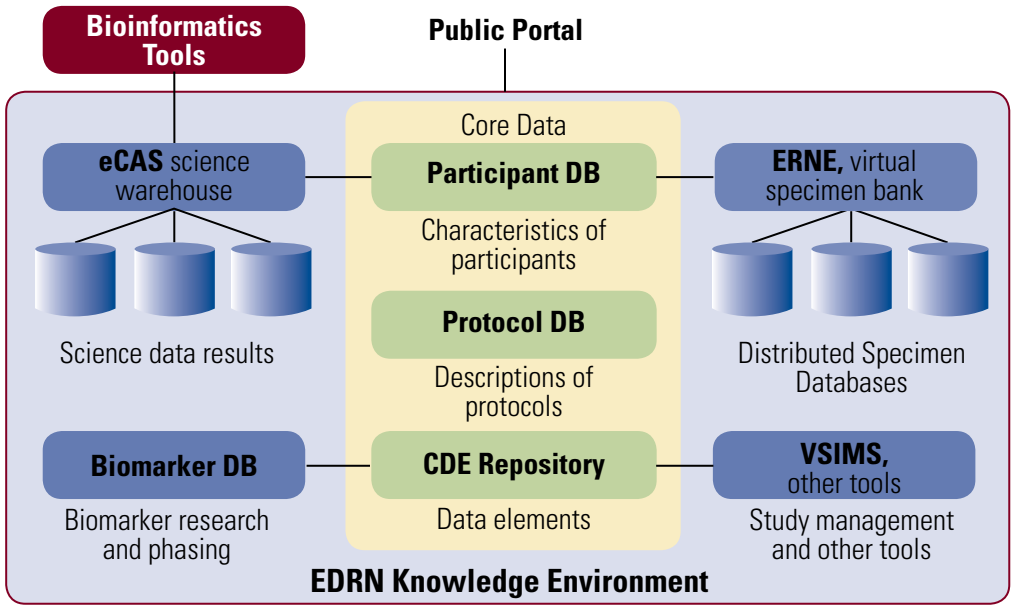
The National Aeronautics and Space Administration's (NASA) Jet Propulsion Laboratory (JPL), the Data Management and Coordinating Center (DMCC) and NCI have all played key roles in developing the informatics systems for EDRN. The first application developed for the Network focused on providing a common informatics framework for accessing heterogeneous biospecimen repositories located at participating sites across the United States. As the infrastructure evolved, the core principles of building services that integrate general client applications with heterogeneous, distributed data resources have not changed.

Recognizing the need to build an effective knowledge system where biospecimens, scientific data, study specific data and biomarker data can be captured, accessed and shared at a national level via a transparent, grid-type architecture, the Network focused on addressing five critical informatics goals:

(1) defining an information model for describing the EDRN problem space;
(2) enabling all components of the knowledge system to be distributed;
(3) providing software interfaces for capture, discovery and access of data resources across the knowledge system;
(4) providing a secure transfer and distribution infrastructure to meet United States federal regulations for data sharing; and
(5) providing an integrated portal environment across the distributed EDRN.

Recently, EDRN made significant advances in extending and deploying to Network centers the informatics framework to support the management of biomarker information, including specific annotations of markers, capture of science data and management of the study-specific information along with a scientific portal for accessing this information. A major new release of this capability integrated with a science portal was deployed in 2007. Figure 7-3 shows the architecture and components of the EDRN knowledge system.

Figure 7-3. Architecture and Components of the EDRN Knowledge System



Source: EDRN Informatics Team

The basis for constructing the EDRN knowledge system is the use of common data elements (CDEs). These provide a common language that future studies can use and enable consistency across institutions collecting data. The Network continues to curate CDEs for managing the EDRN's data assets in a consistent way across informatics systems and Network studies.

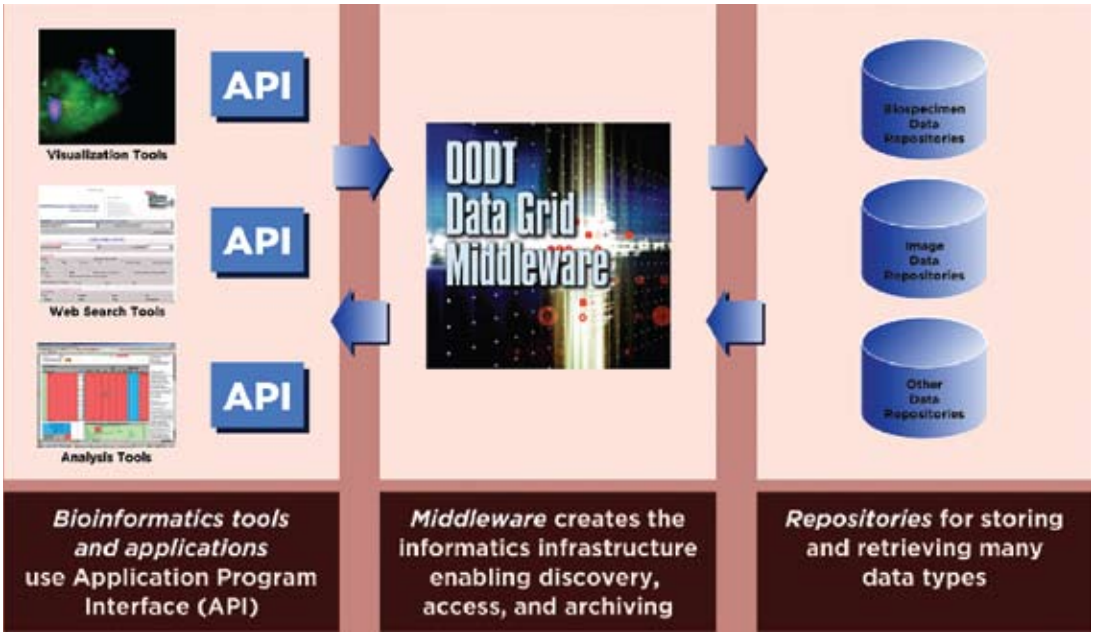
Informatics experts have also been careful to ensure that any data shared is compliant with federal privacy and security regulations including the Health Insurance Portability and Accountability Act (HIPPA). This requires that certain identifiers be removed to protect the confidentiality of the patients described by the research data. In addition, careful attention was applied to the national informatics infrastructure to ensure secure data transmission.

Informatics Infrastructure Connects and Builds Databases

The informatics infrastructure for the EDRN knowledge system is based on a distributed software framework developed at JPL called the Object-Oriented Data Technology (OODT) framework. OODT was selected in 2003 as NASA's Runner-up for "Software of the Year" within the agency. The framework, used to support NASA's planetary and earth science missions, provides a set of software tools capable of both connecting heterogeneous databases together and building new databases capable of archiving data (see Figure 7-4).

The EDRN informatics infrastructure combines the Network CDEs with the OODT software to enable common mechanisms for searching databases located at EDRN-funded research institutions. Because the software is intelligent enough to handle mappings between different database implementations, scientists and other researchers can make discoveries using different data sets produced by different organizations with different meanings, as if they are a single, large repository of knowledge. This means that the software can be configured to fit several different domains that are critical to scientific research, such as biomedicine and space science. A great advantage is that it connects disparate databases and systems together over the Internet without requiring those systems to be re-implemented or modified.

Figure 7-4. EDRN's Informatics Infrastructure is Based on the NASA/JPL OODT Data Grid Architecture.



Source: NASA Jet Propulsion Laboratory

CDEs Produce Interoperability Among Groups

Data architecture is critical to effectively search heterogeneous distributed data systems and to enable correlative science. This structure defines the CDEs and their relationships within the EDRN knowledge environment and enables the interoperability between distributed institutions by providing a common semantic language for communication.

Several standards have been adopted that support the definition of data architecture. ISO/IEC 11179 provides a standard definition for describing data elements. This enables consistency when developing data dictionaries. The ISO/IEC standard recommends that a data element consists of attributes for four key categories: identification, definitional, representational and administrative. EDRN uses ISO/IEC 11179 in conjunction with Dublin Core as a mechanism for developing a minimal set of data elements that must be provided in any data architecture.

EDRN developed data architecture for its knowledge system that provides an over-arching **ontology model** for describing critical cancer data objects. An ontology model is a concept used to represent knowledge in a domain (e.g., management of biomarkers). This model was captured as a set of CDEs, standard data terms and associated values that are critical for enabling data sharing and capture systems.

Each participating institution within the EDRN knowledge system was using the Network CDEs to map their local data models to the knowledge system model in order to provide semantic consistency across the entire system. Specific mapping tools were developed to allow informatics experts to then capture the mapping of local site data models to the EDRN knowledge system model. Attributes of the data element, including permissible values, units, format and data type, were in turn captured and mapped to one another. This enabled the informatics infrastructure software to run a translation function as part of the process of querying and retrieving data from the distributed EDRN institutions.

Biospecimen Data Unified Across Institutions

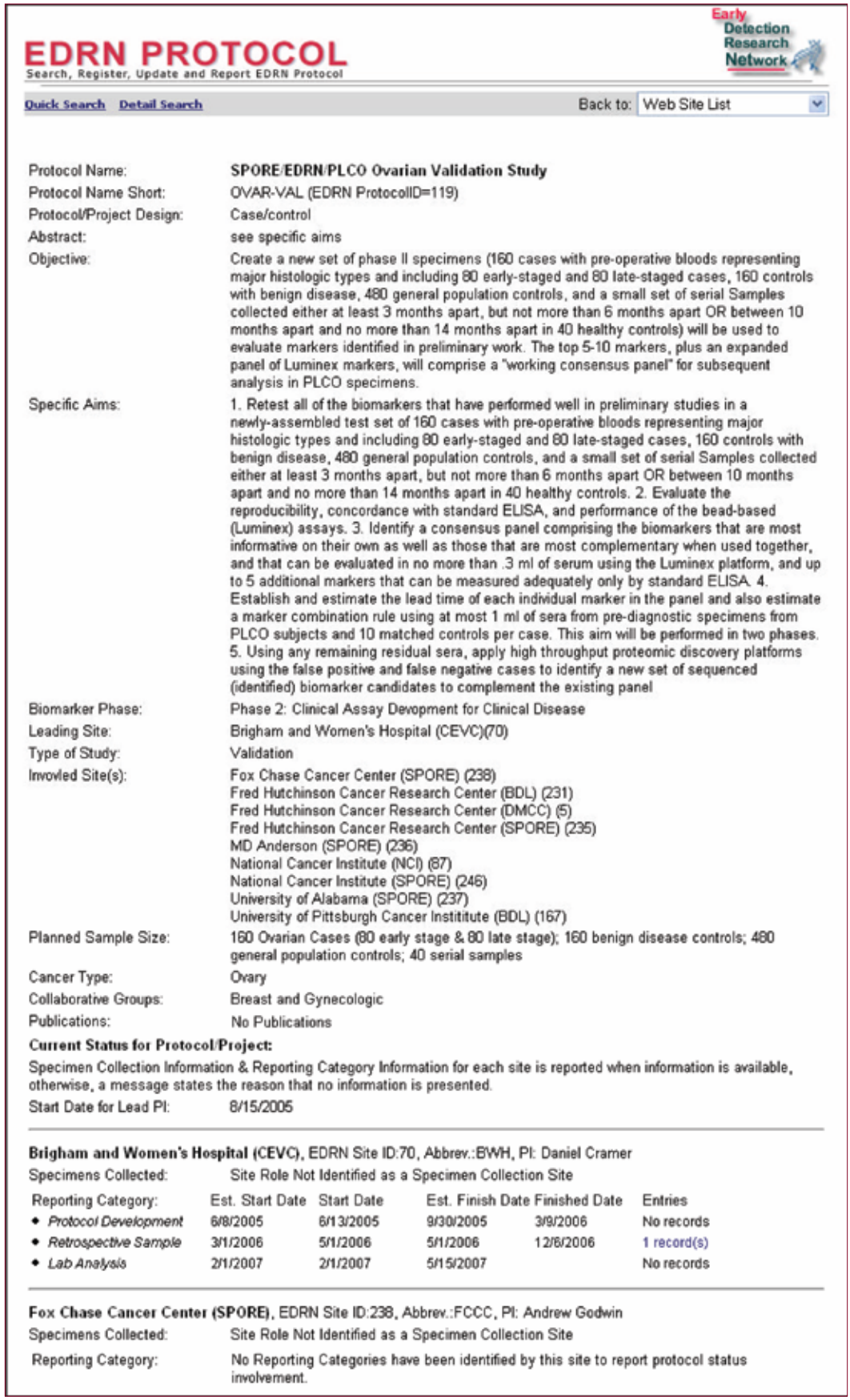
EDRN already deployed the knowledge system to over 10 institutions providing a common web-based client interface called "ERNE" or the EDRN Network Exchange system. ERNE unifies search and retrieval of biospecimen data from all institutions regardless of its location, storage, or differences in the underlying data models. This helps scientists, for example, to locate tissue specimens for breast cancer by searching data catalogs at participating institutions across the country (see Figure 7-5).

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

Figure 7-5. Distributed ERNE Specimen System

Source: DMCC at Fred Hutchinson Cancer Research Center

Figure 7-6. Web Site Screen Capture of EDRN Protocol Management Systems



EDRN PROTOCOL
Search, Register, Update and Report EDRN Protocol

Early Detection Research Network

Quick Search | Detail Search | Back to: Web Site List

Protocol Name:	**SPORE/EDRN/PLCO Ovarian Validation Study**
Protocol Name Short:	OVAR-VAL (EDRN ProtocolID=119)
Protocol/Project Design:	Case/control
Abstract:	see specific aims
Objective:	Create a new set of phase II specimens (160 cases with pre-operative bloods representing major histologic types and including 80 early-staged and 80 late-staged cases, 160 controls with benign disease, 480 general population controls, and a small set of serial Samples collected either at least 3 months apart, but not more than 6 months apart OR between 10 months apart and no more than 14 months apart in 40 healthy controls) will be used to evaluate markers identified in preliminary work. The top 5-10 markers, plus an expanded panel of Luminex markers, will comprise a "working consensus panel" for subsequent analysis in PLCO specimens.
Specific Aims:	1. Retest all of the biomarkers that have performed well in preliminary studies in a newly-assembled test set of 160 cases with pre-operative bloods representing major histologic types and including 80 early-staged and 80 late-staged cases, 160 controls with benign disease, 480 general population controls, and a small set of serial Samples collected either at least 3 months apart, but not more than 6 months apart OR between 10 months apart and no more than 14 months apart in 40 healthy controls. 2. Evaluate the reproducibility, concordance with standard ELISA, and performance of the bead-based (Luminex) assays. 3. Identify a consensus panel comprising the biomarkers that are most informative on their own as well as those that are most complementary when used together, and that can be evaluated in no more than .3 ml of serum using the Luminex platform, and up to 5 additional markers that can be measured adequately only by standard ELISA. 4. Establish and estimate the lead time of each individual marker in the panel and also estimate a marker combination rule using at most 1 ml of sera from pre-diagnostic specimens from PLCO subjects and 10 matched controls per case. This aim will be performed in two phases. 5. Using any remaining residual sera, apply high throughput proteomic discovery platforms using the false positive and false negative cases to identify a new set of sequenced (identified) biomarker candidates to complement the existing panel
Biomarker Phase:	Phase 2: Clinical Assay Devopment for Clinical Disease
Leading Site:	Brigham and Women's Hospital (CEVC)(70)
Type of Study:	Validation
Invovled Site(s):	Fox Chase Cancer Center (SPORE) (238) Fred Hutchinson Cancer Research Center (BDL) (231) Fred Hutchinson Cancer Research Center (DMCC) (5) Fred Hutchinson Cancer Research Center (SPORE) (235) MD Anderson (SPORE) (236) National Cancer Institute (NCI) (87) National Cancer Institute (SPORE) (246) University of Alabama (SPORE) (237) University of Pittsburgh Cancer Instititute (BDL) (167)
Planned Sample Size:	160 Ovarian Cases (80 early stage & 80 late stage); 160 benign disease controls; 480 general population controls; 40 serial samples
Cancer Type:	Ovary
Collaborative Groups:	Breast and Gynecologic
Publications:	No Publications

Current Status for Protocol/Project:

Specimen Collection Information & Reporting Category Information for each site is reported when information is available, otherwise, a message states the reason that no information is presented.

Start Date for Lead PI: 8/15/2005

Brigham and Women's Hospital (CEVC), EDRN Site ID:70, Abbrev.:BWH, PI: Daniel Cramer

Specimens Collected: Site Role Not Identified as a Specimen Collection Site

Reporting Category:	Est. Start Date	Start Date	Est. Finish Date	Finished Date	Entries
• *Protocol Development*	6/8/2005	6/13/2005	9/30/2005	3/9/2006	No records
• *Retrospective Sample*	3/1/2006	5/1/2006	5/1/2006	12/6/2006	1 record(s)
• *Lab Analysis*	2/1/2007	2/1/2007	5/15/2007		No records

Fox Chase Cancer Center (SPORE), EDRN Site ID:238, Abbrev.:FCCC, PI: Andrew Godwin

Specimens Collected: Site Role Not Identified as a Specimen Collection Site

Reporting Category: No Reporting Categories have been identified by this site to report protocol status involvement.

Source: DMCC at Fred Hutchinson Cancer Research Center

Information System for Study Management

The Validation Studies Information Management System (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.

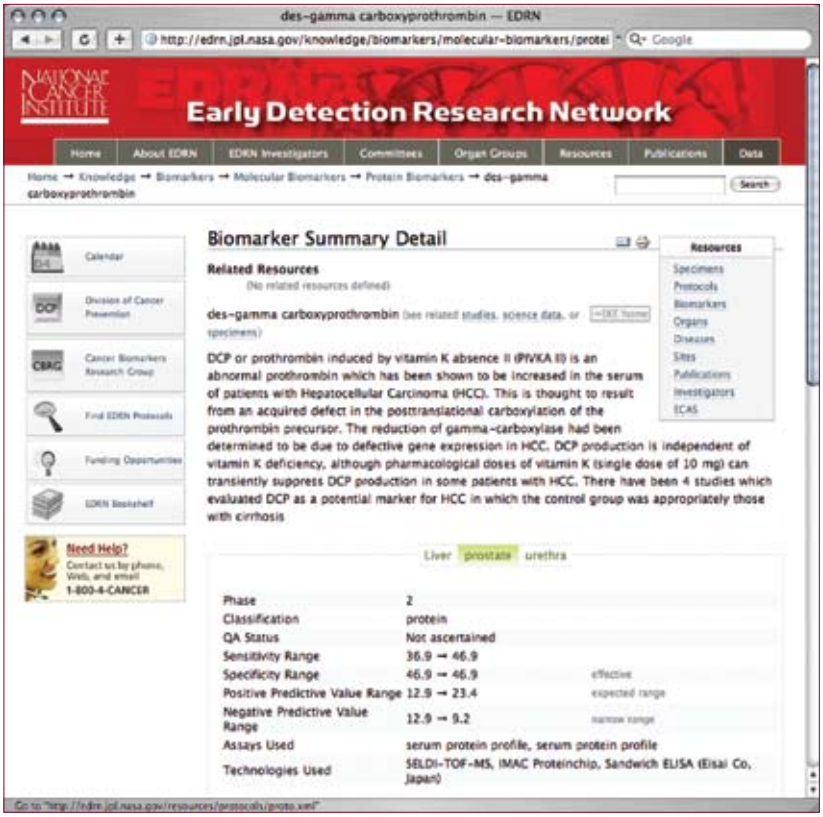
EDRN designed a secure, web-based system that includes the main components needed for capturing and preserving the necessary metadata and data objects that integrate into the overall knowledge system architecture. These components include protocol management tools (see Figure 7-6), communication tools, a data collection and processing system and a specimen tracking system. All are based on having a robust data architecture. Information maintained in the system is secure and stored separately for each multisite study, allowing multiple protocols to be coordinated centrally through the same data management system.

Biomarker Data Management

Biomarker data management involves managing a database for tracking biomarker research, including collection of such data as phase of development, 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. Figure 7-7 shows an example biomarker study.

Figure 7-7. Web Site Screen Capture of Biomarker Study Tracking Example



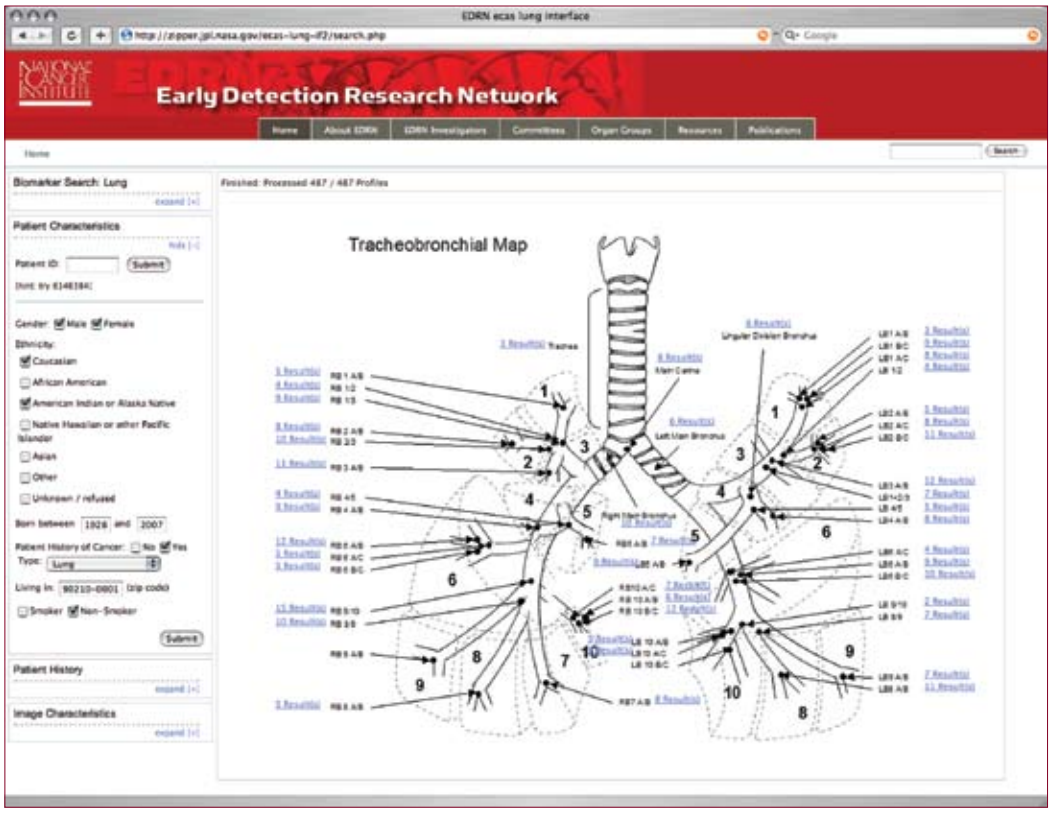
Source: EDRN Informatics Team

Data is captured consistently using the same set of CDEs and therefore, applications can interoperate and automatically correlate information. This forms the logical EDRN Knowledge System. For example, a biomarker tracked in the biomarker database can link to a cell count in a specimen record in ERNE; the result of an analysis can reference science data captured as well. Access to this information through a shared mechanism provides an integrated view of the information within the EDRN enterprise.

EDRN is also establishing a science data warehouse called the EDRN Catalog and Archive System (eCAS). eCAS is a distributed metadata-driven system for the capture, tracking, processing and retrieval of scientific data from biomarker validation studies. eCAS promises to be an invaluable tool that will make it possible to share results, correlate data, discover new biomarkers and much more.

eCAS also will allow for capture and release of public data sets housed at NCI, as well as sharing specific science data from institutions. eCAS is 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. Figure 7-8 below shows a prototype under development within EDRN that allows for the search and discovery of lung images based on regional queries of the bronchial map.

Figure 7-8. Web Site Screen Capture of Prototype Biomarker Atlas for the Lung



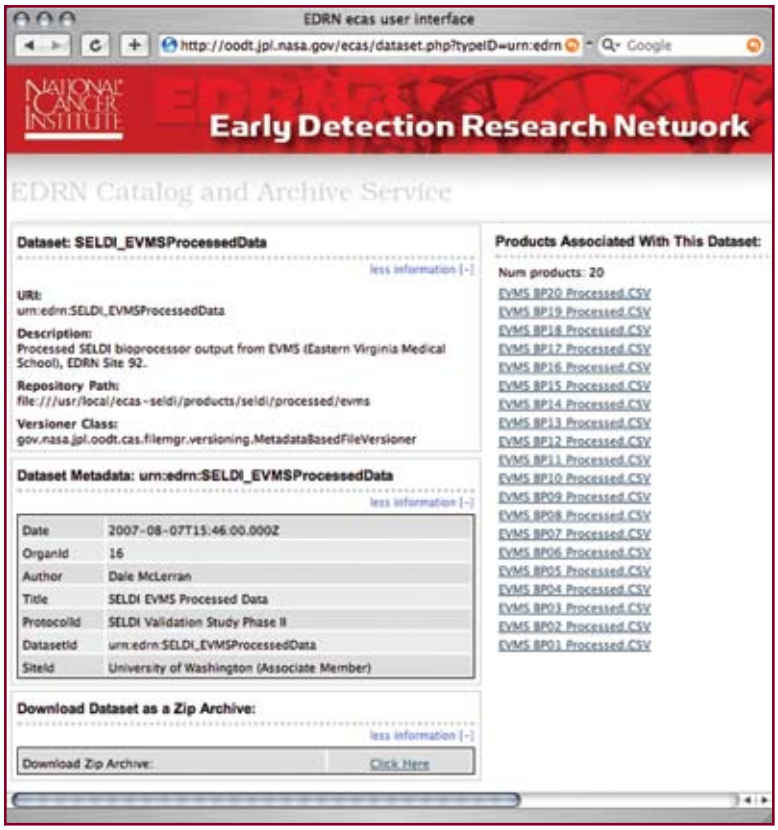
Source: EDRN Informatics Team

Public Information Disseminated through Portals

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 as shown in Figure 7-9. 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.

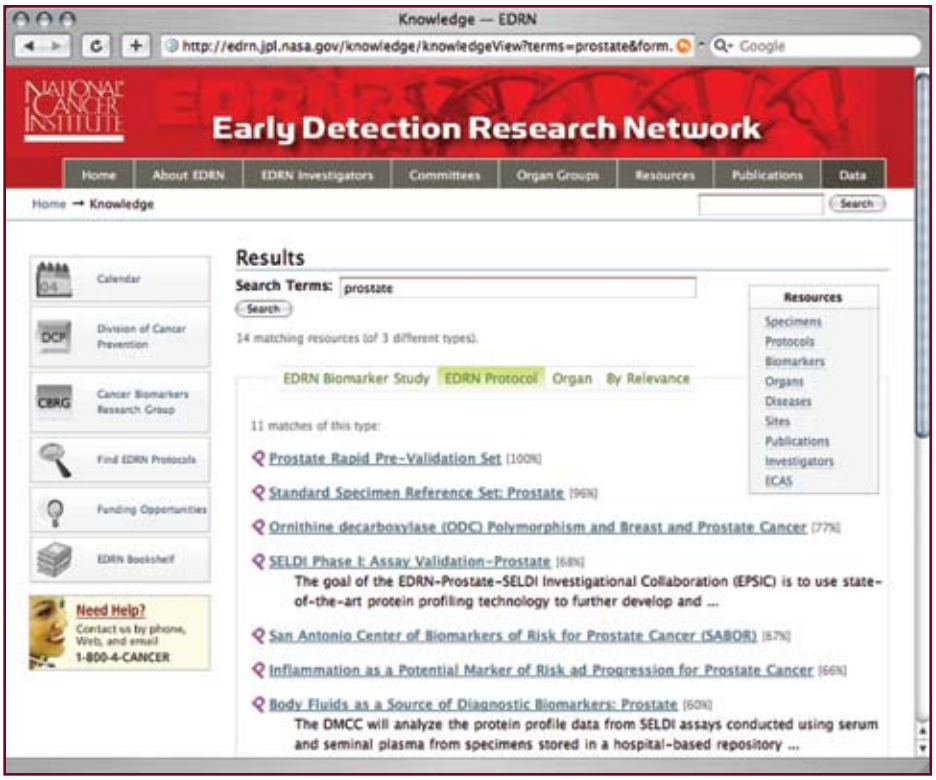
EDRN is preparing to release an upgrade to the public portal that will transform it into a knowledge portal for accessing the science information produced during EDRN studies. The public portal will play a pivotal role by permitting Google-like searching of the EDRN information space, allowing users to navigate the complex set of information available within the EDRN enterprise. Figure 7-10 demonstrates a search of information related to prostate cancer within the EDRN.

Figure 7-9. Search for Science Data Using the EDRN Public Portal



Source: NASA Jet Propulsion Laboratory

Figure 7-10. Results from an Online Search for EDRN Prostate Cancer Protocols



Source: EDRN Informatics Team

The cancer Biomedical Informatics Grid, or caBIG™, is helping to lead NCI in developing a research informatics infrastructure for scientists. At the same time, 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 aide 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. This 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://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.

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 and improve patient management. ■

Part Three

Investing in Biomarker Research

CHAPTER EIGHT

Business Model

"We are victims of our own success. The sheer number of candidate biomarkers creates an impediment to their further development as it is not easy to recognize those markers that have the greatest potential.… (T)he development of biomarkers would benefit from an organized community effort that allows progression from discovery to validation. A case in point is the National Cancer Institute's Early Detection Research Network, which fosters a collaborative effort and provides access to critically needed standardized reference specimens.… It is time to recognize that developing biomarkers is just as complex as developing drugs and cannot just be done on an ad-hoc basis but through concerted efforts that bring together academia, industry, government and foundations."

Sam Hanash, M.D., Ph.D.
Principal Investigator, EDRN Biomarker Development Laboratory
Fred Hutchinson Cancer Research Center
(from the *Journal of Proteomics Research*)

WHY DO "BIG SCIENCE" proposals that have such great promise, so often require decades from concept to fruition (if they make it to practice at all)? The health care industry, while accounting for more than 13% of the U.S. gross domestic product and growing at triple the rate of inflation, remains a fragmented industry. Perhaps health care can learn from computer electronics, where "big science" is always part of the equation, but so is a business model that drives the translation from "art to part." Part of the problem is the difference in the business models that drive the respective systems.

Health care organizations have generally grown organically, which typically results in structures that are organized along functional "silos," i.e., in areas of expertise where depth of knowledge in one particular area is critical. Such a "horizontal" structure fosters excellent solutions for primary scientific problems.

However, it often generates barriers if knowledge must be shared between silos. In contrast, computer chip manufacturers are organized in a more "vertical" structure. In this structure, formal "hand-off" procedures have been designed to ensure that discoveries in one aspect of chip design and construction are rapidly and efficiently conveyed to others who require the information. This allows for rapid vetting of ideas, quickly culling out the poor concepts and fostering the rapid acceptance of good concepts.

In a vertical design there are a number of focused experts in a single organizational unit, generating rapid discovery of ideas with a primary focus on: coordination of multiple entities; using shared resources; and emphasizing the "hand-offs" between entities.

Novel Mechanism Enhances Collaboration

EDRN promotes a **vertical approach** for conducting biomarker research, whereby biomarkers are developed in Biomarker Developmental Laboratories (BDLs), refined and cross-validated by Biomarker Reference Laboratories (BRLs) and validated in collaboration with Clinical Epidemiology and **Validation** Centers (CEVCs), all within one organization (see Figure 8-1). The focus is in coordinating multiple resources with a goal of minimizing the barriers to the rapid and efficient "hand-off" between entities. One method used for achieving this is a structured set of criteria for assessing the roles and clinical significance of each newly discovered biomarker, along with criteria and strategies for judging biomarkers in relationship to one another (see Figure 8-2).

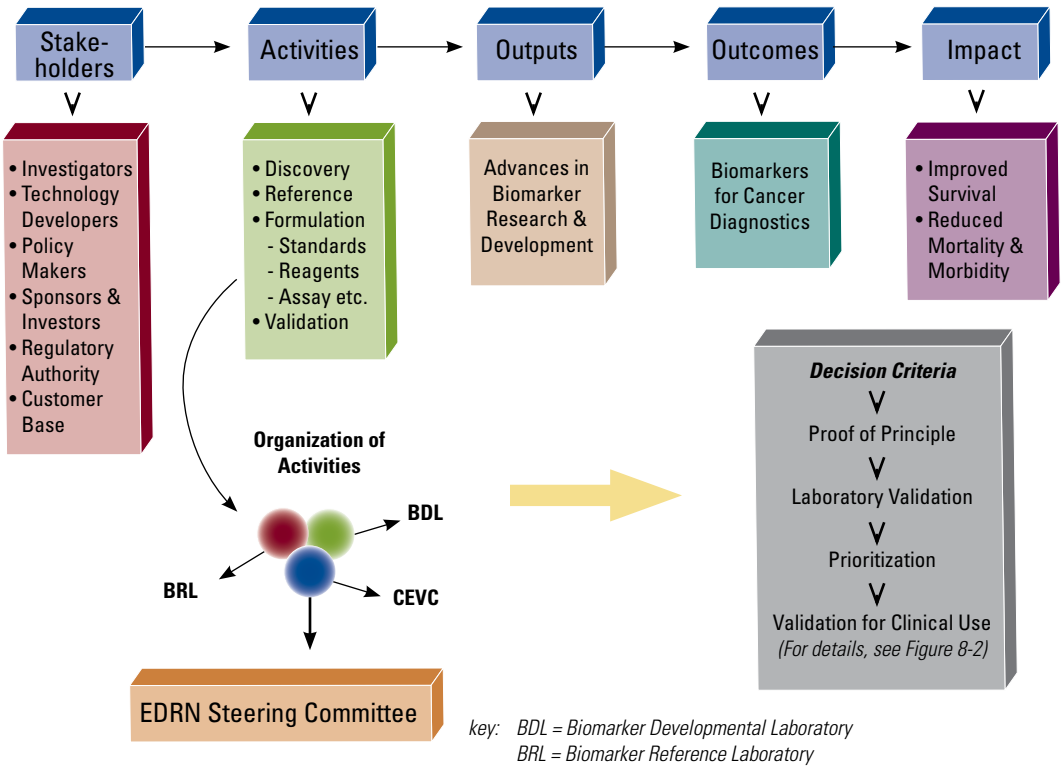
This is in contrast to a **horizontal approach**, which may result in rapid discoveries of many biomarkers by participating laboratories, but limits advancement of the biomarkers to validation while increasing duplication and reducing potential synergies across disciplines.

In either model, particularly in health care, one must consider the influence and interests of constituents or forces that can either drive or hinder the process of forward movement. EDRN developed methods, policies and procedures for relating with each of these major constituent groups in an approach adapted from the *Harvard Business Review*.

Figure 8-1. The Vertical-Approach Business Model followed by EDRN

Any business model is defined by the organization's clients, core values, inputs, expected outcomes and what impact is projected in business.

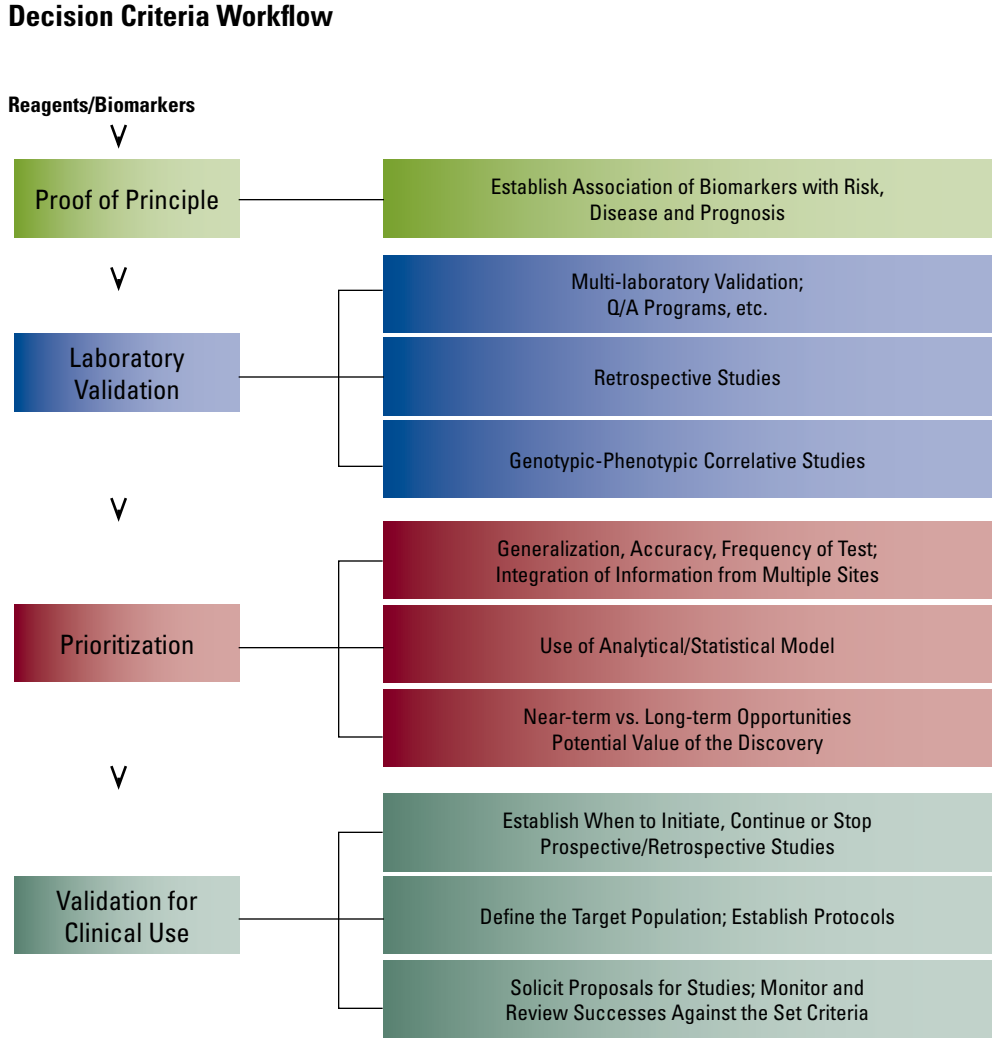
EDRN Business Model



key: BDL = Biomarker Developmental Laboratory
BRL = Biomarker Reference Laboratory
CEVC = Clinical Epidemiology & Validation Center

Adapted from the *Harvard Business Review*

Figure 8-2. Decision Criteria for Judging Relationships and Strategies in Biomarker Development and Fruition



Source: NCI EDRN

Investigators

Players or stakeholders are groups or individuals with a stake in the results of the EDRN's efforts. The biomarker research enterprise has many stakeholders with substantial resources and power to influence the outcome and adaptability of biomarker-based clinical applications. These include basic science researchers, clinicians, health care professionals, policy makers, regulators and the groups and agencies that fund research. This diverse assemblage can have competing priorities and agendas; any successful business model must identify a common theme around which all can work synergistically. EDRN joins stakeholders around the hub of **translational research**. The Network structure is defined by a mission based on inclusiveness, openness, coordination and cooperation among normally disparate groups.

Sponsors and Investors

Due to the long investment time needed to discover, develop and use biomarkers in clinical application, investors in biotechnologies and pharmaceutical companies are less inclined to support biomarker diagnostics research. Progress in the field has been slow due to a lack of sustained funding. EDRN is one of the few mechanisms in use to jump-start the process. Funding is a major force shaping the Network,

without which this model is not viable. With sustained funding in place, EDRN established relationships with industry, foundations and international consortia, to generate and share precompetitive and prevalidation data on biomarkers and to sponsor **validation** projects that develop diagnostic biomarkers.

Sponsors and investors need to take a long-term view of the development cycle of EDRN. The Internet, for example, was an Advanced Research Projects Agency project of the U.S. Defense Department under construction for years before it generated today's World Wide Web. Without sustained funding by sponsors, the necessary infrastructure of the Internet would never have been developed.

The funding mechanism to support this business model is derived from the principles and concepts found in other sectors of the national economy. In these sectors, "production," be it the creation of a restaurant meal, the building of a computer chip or the flying of passengers from one destination to another, is accomplished through a series of independent, but synergistic units that work together to create the end products or service. For example, a restaurant may have a wine steward, dessert chef and head chef, all of who must be coordinated to "produce" the best meal for the consumer. Similarly, the various components of EDRN are coordinated to assure that good science rapidly and efficiently conveys from one aspect of development to the next.

Policy Makers and Regulators

Existing policies and regulations on biomarkers in health care are not clearly delineated and continue to emerge. EDRN is in an excellent position to work with regulators, such as the Food and Drug Administration (FDA), to clearly define approval requirements for biomarker tests and molecular diagnostics. Also, NCI has ongoing discussions with the Centers for Medicare and Medicaid Services for covering nine NCI-sponsored clinical trials of colorectal and other cancers.

Technology Developers

Stiff competition exists among technology developers. Many are not receptive to participating in technology comparisons and, not surprisingly, frequently resist working with their rivals. EDRN overcame this obstacle by bringing in developers at the outset of studies and building confidence in favor of the proposed study that provides a "win-win" situation for all stakeholders.

Customer Base

The cancer community has an abundance of engaged and empowered individuals. Organ-specific advocacy groups increase awareness of cancer and vigorously support sustained funding for the various national and international programs. Many professional societies, such as the American Cancer Society, American Association for Cancer Research, American Society of Clinical Oncologists and others, lobby for research funds. EDRN investigators link with these societies on a regular basis and invite their leaders to the EDRN-sponsored meetings and conferences. The Network recognizes and leverages the influence of the societies, consumers and interest groups in promoting biomarker research.

Increasingly, informed stakeholders are demanding accountability from networks or consortia perceived to be favored over individual-based science. EDRN is at the forefront of adopting critical benchmarks for measuring productivity and accountability in its business model. The model promotes collaboration, discourages "Zombie projects" and rewards team-science. "Zombie Project" is a term used in project management for projects that continue endlessly without a closure and delivering on their promises.

EDRN accomplishes this task through various laboratories and centers under the administrative guidance of a Steering Committee and NCI program staff. Together they monitor performance and coordinate incentives and rewards for collaborating investigators through novel funding approaches. Proficient administrative tools track projects and ensure completion. Another element of accountability ensures

that investigators meet their stated goals and develop collaborations with other investigators before a specific set of funds is released. Monies released from set-aside or special core funds are provided only if the investigators' performance is at an acceptable level as judged by the site visit team, EDRN Steering Committee and NCI program staff. These set-aside funds (20% of individual grants) and Core Funds are utilized to promote team science and some "Big Hairy Audacious Goals," as well as to accelerate the discovery and validation process when additional resources and expertise are needed. "Big Hairy Audacious Goals" (BHAG) refers to using ambitious, even outrageous goals to motivate people and focus them toward concrete accomplishments.

In the EDRN funding process, individual grantees apply for the release of set-aside funds from their grants for collaborative projects. The request is reviewed and approved by the EDRN Executive Committee and then read by other EDRN investigators with appropriate expertise. A request for core funds undergoes a rigorous two-step review that involves submission of a three-page pre-application, followed by a full application that is evaluated by two external reviewers and the EDRN Executive Committee. Associate Membership is used to seek additional expertise not currently available within the Network. Applications for Associate Membership are reviewed online and by a Standing Review Committee.

This adaptive funding approach ensures that elegant, novel ideas win and are supported in a timely manner. Additionally, the management oversight provides necessary "checks and balances" to utilize scarce resources wisely and enforce benchmarks for timely project conclusion.

Associate Members and Their Contributions

Category A

Members submit basic or translational research consistent with EDRN priorities. They bring in new ideas and proposals for biomarker discovery.

Category B

Members contribute to Network priorities by sharing available technologies, contributing specimens, providing high-risk registries, cohorts and other resources. Funding can be applied for annually.

Category C

Members include scientists, clinicians, patient advocates and ethicists, who participate in EDRN workshops and conferences and Collaborative Group meetings, but do not receive EDRN funding or support for travel expenses.

EDRN Business Model Promotes Collaboration

While NCI leads the nation's investment in cancer research, it is not the nation's sole contributor in the fight against cancer. Critical contributions are made by other government agencies, academic and charitable organizations and private industry. Coordinated efforts of the parties can result in productive partnerships where the various groups, collectively and individually, each contribute to forwarding progress.

EDRN established numerous platforms to facilitate and enable cancer research. Such platforms create an infrastructure for translational and clinical research, providing unique resources, reagents, information exchange and the critical mass of researchers, facilities, technologies and disciplines. The goal and demonstrated talent of NCI is to make investments that maximize the opportunities for progress.

Industry plays a distinctive role in bringing the products of the nation's investment in research from development to clinical use, as well as serving as a valuable contributor in all other stages of the research and discovery process. Industry requires scientific infrastructure that may overlap the needs of ongoing research efforts supported by NCI. Costs and management of investments to support developing infrastructure frequently

exceed the capabilities of any one company and do not require proprietary access. Again, consider the Internet, where the development cost was well beyond the reach of any single firm but not beyond the collective effort of public-private partnerships.

Both EDRN and industry benefit from a mechanism that allows formal partnering in development and operation of necessary infrastructure. Partnerships strengthen collaborations by creating the vehicle for industry to interact with and, where appropriate, co-fund development of infrastructure with NCI. Industry benefits by having an opportunity to access new resources, expertise, databases and reagents resulting from NCI-coordinated infrastructure investments. NCI benefits by having access to the strengthened capabilities of industrial partners, which are often essential for bringing the products of research investments to the American public: products such as industry expertise and technology; an expanded investment base to build critical infrastructure for research; and expanded scientific scope for the scientific priority-setting process.

Significant ongoing activities within EDRN are poised to accelerate the discovery process and regulatory approval. Not only does it take many years to bring biomarkers to clinical use but development requires a sizeable dollar investment and infrastructure-related resources. Both private sectors and government institutions face regulatory hurdles, but their investigators together can help regulators learn from researchers' concerns and suggestions to improve the review process.

Historical Collaboration with Industries

Diagnostic firms work with EDRN to help accelerate the discovery, evaluation and validation processes. Some organizations are listed in Table 8-1.

EDRN-Gordon Research Conference

EDRN sponsors the Gordon Research Conference (http://www.grc.org) on "New Frontiers in Cancer Detection and Diagnosis" every other year to emphasize that the accurate detection of early stage cancer is critical to improve patient care. Progress must be made in the development of cancer-specific interventions to avert invasion, metastatic dissemination and subsequent, advanced disease. The conference brings together junior and senior physicians and scientists with expertise in basic, translational and clinical oncology and experts in computational biology and informatics.

The conference setting provides an opportunity and venue for intense discussion and evaluation of cancer research; for establishing the merit of research priorities; and for advancing the field through newly forged collaborations. In an atmosphere focused on uniting biology, oncology and technology, physicians and scientists work cooperatively to cultivate new avenues of research and reveal potential clinical applications to improve patient care.

Dialogue with the Food and Drug Administration

EDRN is in a unique situation to liaise between industry and government and regularly consults FDA. Two such meetings with FDA scientists, EDRN investigators, NCI scientists and diagnostic firms were the EDRN-FDA Education Workshop at the National Institutes of Health, February 15, 2007; and the Joint NCI-FDA Workshop on Research Strategies, Study Designs and Statistical Approaches to Biomarker Validation for Cancer Detection and Diagnosis, July 23-25, 2004.

Collaboration with Foundations

EDRN collaborates with the Canary Foundation on ovarian cancer and the Lustgarten Foundation on pancreatic cancer. The founder and CEO of the Canary Foundation agreed that investigations supported through Canary will consult on validation needs and propose validation of a biomarkers panel through EDRN. Lustgarten is consulting EDRN on developing high-quality monoclonal antibodies through a Request for Application to be issued by the Foundation and reviewed using the EDRN online review system. The antibodies will likely be stored at NCI-Frederick and managed and distributed by EDRN.

Table 8-1. Some EDRN Collaborations with Diagnostic Firms

ENTITY	CONTRIBUTION		VALIDATION STUDY
	Reagents	**Assay**	
Eisai, Japan	Yes	No	DCP for Hepatocellular Carcinoma
Wako	Yes	Yes	AFP, AFL-L3 for Hepatocellular Carcinoma
Gen-Probe	Yes	Yes	Assay for PCA3; Fused transcripts (TMPRSS2-ETV1 and TMPRSS2-ERG) for Prostate Cancer
Abbott	ELISA FISH Probe	Yes	TIMP-1 for Colon Cancer and LOH for Esophageal Cancer; Chromosomal aberrations for Lung Cancer
Fujrebio	Yes	Yes	SMRP in Mesothelioma; HE-4, CA-125 and CA72.4 antibodies for Ovarian panel
Diadexus	Yes	Yes	B7-H4, Spondin and DCR3 for Ovarian marker panel
Milagen	Yes	Yes	Antibody panel for several cancers
LabCorp	Yes	Yes	Ovarian panel
OncoMethylome	Yes	Yes	GSTP-1 for Prostate Cancer
Beckman-Coulter	Yes	Yes	% proPSA for Prostate Cancer
Meso Scale Diagnostics	Yes	Yes	Validating platform for antibody array for Ovarian Cancer

Source: NCI EDRN

Business Model Stimulates Innovation and High-Risk Projects

The phrase BHAG ("Big Hairy Audacious Goal") was proposed by James Collins and Jerry Porras in their article "Building Your Company's Vision," *Harvard Business Review*, (1996) Vol. 74, Iss. 5, pp 65-77.

"A true BHAG is clear and compelling, serves as unifying focal point of effort and acts as a clear catalyst for team spirit," according to their research. "It has a clear finish line, so the organization can know when it has achieved the goal; people like to shoot for finish. Such a concept has been used by many big industrial houses, such as Boeing, IBM, Motorola, etc."

EDRN uses a similar concept for promoting multi-institutional, multidisciplinary projects, such as the EDRN-Human Proteomics Organization (HUPO) collaboration on the Plasma Proteome Project and the EDRN-NIH Women's Health Initiative Project on discovery of colon markers. In these collaborations, a team is formed for a specific project and is dissolved once the project is completed. The team has clearly defined goals, timelines, milestones and closure clauses.

This approach rapidly identifies the outcome of the mission and lends its support either for continuation or dissolution in a timely manner.

Over 7,000 articles are published per year on potential biomarkers, yet the FDA approves only approximately one marker every other year. The problem is clear: there is an enormous gap between the development of potential biomarkers and the conversion of the beneficial ones into approval. An additional gap exists between FDA approval and use in general practice. This raises the "BHAG" of EDRN: to convert this heterogeneous mixture into a seamless, fine-tuned network of systems that can significantly, measurably and dramatically reduce the time between discovery and widespread use of critically important biomarkers in the oncology community.

As the Network strives to sail across the knowledge stream—navigating from gathering data, to sorting information, to accumulating knowledge and, finally to translating it all into usable products—a business model should be prepared to mitigate the many potential obstacles and dangers inherently associated with the continuum from discovery to translation to biomarker-based diagnostic assays.

As discussed earlier, all stakeholders must work together on today's tremendous opportunities, particularly on those technologies that will accelerate biomarker discovery and validation and lead to greater diagnostics for patients.

The vertical approach business model presented here allows EDRN to operate within a "forecast, prevent and manage" paradigm. The paradigm includes, but is not limited to, the following aspects:

- Cancer will be forecast on the basis of clinical and biological profiling.
- Institutional investment in cancer prevention will increase.
- Early cancer detection will be monitored through regular, inexpensive biomarker tests.
- Genetically defined subtypes of disease will be identified and personalized care will be offered.

Business models for industry often perform poorly and the companies fail. Manufacturing sectors have evolved their business models to meet the challenges of the time. Large biomedical science enterprises, however, never had well defined models and, therefore, precedents are lacking. EDRN's model has the flexibility to evolve and adapt to needs as Network collaborators gain more experience, learn about new challenges and obstacles and experience both setbacks and successes. ■

EDRN Investigator Team Receives Team Science Award From the American Association for Cancer Research

EDRN principal investigator Arul Chinnaiyan, M.D., Ph.D. and his team from the University of Michigan SPORE were awarded the Inaugural Team Science Award at the Centennial American Association for Cancer Research (AACR) Annual Meeting for their work on gene fusion in prostate cancer. This award has been established by AACR and Eli Lilly and Company to recognize the growing importance of interdisciplinary teams in the translation of scientific research discoveries into clinical and diagnostic cancer applications. Team members also included Ken Pienta, M.D. (SPORE grantee), James Montie, M.D., John Wei, M.D., and Mark Rubin, M.D.

Their major accomplishments are:

- The discovery of gene fusion (TMPRSS2-ERG, TMPRSS2-ETS; *Science* 310: 644, 2005) in prostate cancer;
- The use of DNA microarrays to develop a molecular signature for prostate cancer (*Nature* 412:822, 2001) and linking of the Polycomb Group Protein and histone methylatransferase EZH2 to solid tumors (*Nature* 419: 624, 2002/ PNAS 100:11606, 2003);
- Autoantibody signatures of prostate cancer (*NEJM* 353:1224) and integrative molecular approaches to study molecular alterations in cancer (*Cancer Cell* 8:393, 2005 *Nature Genetics* 37:579, 2005);
- The team, along with others, was among the first to discover AMACR as a tissue biomarker of prostate cancer (*JAMA*, 287:1662, 2002);
- The Chinnaiyan Lab, through EDRN, is attempting to validate the non-invasive detection of prostate cancer gene fusions in urine.

CHAPTER NINE

Evaluating Biomarker Progress in Translational Research

"Translational research requires cooperative expertise at the clinical, epidemiological and basic scientific levels. If you lack any of these, as all programs excepting the EDRN currently lack, then the research will not be successfully transferred to the clinic. My group is interested in having success at the clinic via biomarker discovery."

William Grizzle, M.D., Ph.D.
Principal Investigator
EDRN Biomarker Development Laboratory
University of Alabama at Birmingham

REDUCTIONS IN CANCER mortality are primarily due to early detection and risk reduction behaviors. NCI established the Early Detection Research Network (EDRN) as a vertically integrated environment to discover and validate **biomarkers** for both the early detection of cancer and for cancer risk assessment. Throughout EDRN's existence, NCI advisory groups, such as the Board of Scientific Advisors (BSA), the National Cancer Advisory Board (NCAB), the **Translational Research** Working Group (TRWG) and other key working groups, supported the Network's model for translational research.

Management through Quantifiable Metrics

EDRN recognizes a need to develop metrics that might be tracked and captured more easily by methods other than those originally developed for the program (e.g., annual progress reports and site visits). The informatics system developed with the National Aeronautics and Space Administration's (NASA) Jet Propulsion Laboratory (JPL) became fully operational in summer 2007. It provides the types of metrics suggested by the TRWG, such as charting milestones and goals, with a system of incentives for moving quickly from Phase 1 to Phase 2 of biomarker development (see Chapter 1). Such progress is being tracked, but the new informatics system allows a greater capacity to monitor progress within individual grants and across the EDRN network and is expected to vastly improve the quantity and quality of metrics. To date, the primary process for evaluating metrics involved the annual written progress report by EDRN members, teleconferences and site visits.

Biomarkers

The most important overall metric for EDRN is the number of biomarkers that have moved forward into **validation** (see Chapter 6).

The Network created strong momentum in biomarker development in the past 7 years. In this type of research, it is expected that there will necessarily be many more markers in the discovery phase (Phase 1) of development than in prevalidation and validation phases (Phases 2 and 3). EDRN has over 120 biomarkers in Phase 1, 27 in Phase 2 and two in Phase 3.

Publications

The number of peer-reviewed publications by EDRN-funded investigators is an important metric to illustrate progress. More than 460 manuscripts have been produced by EDRN investigators and NCI program staff. A list of key collaborative publications appears in Appendix I. Publications produced by two or more EDRN laboratories or with industry show EDRN meeting the key goal of fostering collaborative work.

Conferences and Workshops

As an organization based on a nationwide network of collaboration, EDRN emphasizes participation in Network-sponsored activities, including workshops and conferences. Absent members are considered at risk for exclusion from the program.

Program Evaluation

Metrics for programmatic evaluation, applied to individual laboratories and centers, include quality assurance. These members are also questioned about innovations and future plans, which are vital to ensuring the Network's ability to adapt to changing technologies and scientific progress. Each component of EDRN—Biomarker Developmental Laboratories (BDLs), Biomarker Reference Laboratories (BRLs), Clinical Epidemiology and Validation Centers (CEVCs) and the Data Management and Coordinating Center (DMCC)—established a list of programmatic evaluation metrics. Metrics used by each component are listed below.

- BDL—Biomarkers identified; biomarkers forwarded to CEVCs or BRLs; biomarkers added to early detection or risk-assessment panels; and biomarkers used in chemoprevention clinical trials.
- BRL—Assays performed; number of samples; types of samples; quality control of samples; number and type of developmental projects approved; and use of common data elements (CDEs).
- CEVC—Numbers of samples collected; types of samples; sources of samples; numbers of samples provided to BDLs and BRLs; amount of set-aside funds released; and number of requests for release of developmental funds.
- DMCC—Standards of informatics support; types of informatics; quality-control procedures; development of network-wide communication systems; network-wide communications for data and specimen sharing; and statistical methodology development.

Collaborative Metrics

Collaborative metrics measure how well the EDRN is developed and maintained. Metrics for this area are evidenced by data on the number of EDRN sites collaborating with other EDRN sites; the number of collaboration projects; the number of joint peer-reviewed publications; the number of BDLs that have requested the release of restricted funds; and the number of sites that have collaborated with CEVCs, BRLs, or BDLs. Participation in EDRN can be measured by the number of committees, working groups and task forces attended by members; special projects completed; use of EDRN CDEs for data capture and sharing; data and specimens shared; and willingness to streamline technology transfer.

Outreach Metrics

EDRN outreach, vital to the continuation of the program, speaks to the health of the existing Network. Measures include the number of new Associate Members, the amount of Chair funds allocated to new Associate Members and the number of applications for Chair funding. Actions to broaden outreach to professional organizations provide an opportunity to recruit new EDRN Associate Members and to strengthen EDRN collaborations within the professional community. Participation includes, for example, involvement in the American Association for Cancer Research (AACR), the American Society of Clinical Oncology (ASCO) and the American Society of Molecular Pathologists (ASMP).

External Committee Evaluation

NCI's Division of Cancer Prevention instituted an external committee composed of Bernard Levin, M.D., of the University of Texas M. D. Anderson Cancer Center, Kenneth Cowan, M.D., of the University of Nebraska Medical Center, Barry Kramer, M.D., of the National Institutes of Health, Brian Reid, M.D., of Fred Hutchinson Cancer Research Center and Arnold Kaluzny, Ph.D., of the University of North Carolina at Chapel Hill, to evaluate the EDRN program. They found that the "overall outcomes and accomplishments of EDRN to date have been well worth the cost." According to the committee:

Given that biomarker development must begin at the earliest stage of discovery, EDRN's single accomplishment is having produced a developmental pipeline that provides standardized procedures and measurable milestones…There have been a large number of biomarkers (101 to date) that have entered the pipeline; progress is moving rapidly and is facilitated by an organized management and informatics system that is both adaptable and efficient. This comprehensive list illustrates the breadth and depth of the program across cancer sites and cancer types.

Metrics developed in the past 5 years are maturing with the new database being developed by EDRN and JPL… This database will facilitate methods of improving the program's cost-effectiveness and will provide intensive monitoring of EDRN activities. EDRN challenged the very culture of academic research by its emphasis both on team science as well as close attention to milestones of biomarker development, a rigorous process not familiar to many academic environments. This challenge, though more difficult in the early years of the program, is well on the way to being overcome.

Progress in Translational Research

EDRN 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.

The way EDRN is organized provides flexibility to respond in a timely fashion to new opportunities; there are no barriers that prevent EDRN from responding to changes in research priorities. EDRN made a major educational contribution to the research community at large by providing criteria and new standards for validating biomarkers via published validation study protocols designed by EDRN investigators. The Network continues to address a multiplicity of needs in discovery, validation, tissue collection, informatics, public sector collaboration and engaging academia and the private sector. Fulfilling the expectations for rapid discovery and validation of cancer biomarkers requires continued and sustained investment in biomarker research (see Chapter 10). The process of bringing new biomarkers to the clinic faces challenges similar to the process of bringing new pharmaceuticals to the clinic. But with the current infrastructure in place, these expectations can be realized in the near future.

EDRN adheres to principles and practices of effective management that meet the standards and ideals recommended by the TRWG, some of which are highlighted below:

- **Integrated Organizational Approach**
  EDRN stays abreast of similarly funded programs and rather than duplicate efforts, proposes collaboration and coordination with other NCI biomarker research efforts. The Network operates a secure website for member-investigators (for example, to obtain access to the catalogues of specimens across EDRN sites). There is significant interest in this data from outside investigators and the specimen information on the secure site is being moved to the public site pending IRB review and approval. EDRN embarked on creating standards for methods, tools, specimens and technologies and sought consensus on best practices.

EDRN Biomarker Development Laboratory Achieves Model For Integrative Analysis Through Public-Private Partnership and Metabolomic Profiling

The University of Michigan EDRN Biomarker Development Laboratory (BDL) received grant money from NCI to both extend a collaboration with Harvard University and to establish a new collaboration with Metabolon, Inc., of Raleigh, N.C. This unique public-private partnership focuses research on metabolomic profiling in the study of human disease.

The Michigan BDL was focused on a number of prostate cancer biomarkers, including TMPRSS2-ETS gene fusions and cancer autoantibody signatures. Metabolon is a leading company in the area of unbiased metabolomic profiling of biological specimens.

The overall goal of the project is to integrate genomic, proteomic and metabolomic data to better understand prostate cancer progression and to nominate new biomarkers. Multiple complex molecular events characterize prostate cancer initiation, unregulated growth, invasion and metastasis. Distinct sets of genes, proteins and metabolites dictate prostate cancer progression. Deciphering the molecular networks that distinguish organ-confined prostate cancer from metastatic disease may lead to the identification of biomarkers of invasion and disease aggressiveness.

Although gene and protein expression have been extensively monitored to understand prostate cancer biology, not much is known about the metabolomic profile that represents the distal read-out of this disease pathophysiology. Using a combination of high-throughput liquid and gas chromatography-based mass spectrometry, more than 1,265 metabolites across 262 clinical samples related to prostate cancer (tissue, urine and plasma) were profiled.

The metabolomic profiles derived from tissues were able to segregate benign prostate disease, clinically localized prostate cancer and metastatic disease. Interestingly, these metabolomic profiles revealed increased methylation potential that could drive late-stage epigenetic silencing. Matched transcriptomic data validated the existence of the observed metabolomic alterations. As a reflection of an increase in both the amino acid pool and the methylation potential of the tumor, metabolomic profiling revealed sarcosine as being significantly elevated in metastatic prostate cancer. Increased sarcosine levels were validated in an independent set of metastatic prostate cancer tissues and invasive prostate cancer cell lines. Thus, metabolomic profiling may serve as an important complement to other multi-dimensional molecular approaches to study human disease.

The team included: Principal Investigator Arul Chinnaiyan, M.D., Ph.D. University of Michigan Medical School; collaborators from the University of Michigan, Arun Sreekumar, Ph.D, John Wei, M.D., Debashis Ghosh, Ph.D., Rajal Shah, M.D., Subramaniam Pennathur, M.B., B.S., Gil Omenn, M.D., Ph.D., Laila Poisson, B.S., T. Rajendiran, Ph.D., Xuhong Cao, M.S., Ph.D., K. Shanker, M.S., Bo Han, M.D., Ph.D., Anuradha Giri; collaborators from Metabolon, Inc., Chris Beecher, Ph.D., Alvin Berger, Ph.D., Bruce McCreedy; and collaborators from Harvard University, Marty Sanda M.D. and Mark Rubin, M.D.

- **Designated Funds to Facilitate Promising Translational Research** EDRN was designed from the beginning to support promising translational early detection biomarker projects. Money is set aside from core funds specifically and separately for validation and for discovery. Teams who conduct validation are separate from, but collaborate with, the discovery teams. The opportunity for validation is built into the grant mechanism, with organ site groups responsible and accountable for moving promising markers to validation. Resources for validation are not an obstacle and specimen banks are available through Network collaborations and EDRN reference sets. Decisions on which biomarkers should be validated are guided by the EDRN Executive Committee according to priorities set out for translation of biomarkers.

- **Prioritization Process for Early Translational Research** The EDRN principal investigators comprise the steering committee. Every investigator must also be a member of an organ site group that decides priorities for validation. Each investigator serves on subcommittees or working groups, such as one on cross-cutting technology or one to set EDRN priorities. The decision process described in the manual of operations is transparent. The manual reflects processes voted on by the steering committee. Any investigator from inside or outside the Network can propose a candidate biomarker for validation. Investigators collaborate to create common specimen resources to test biomarkers from the larger scientific community. Decisions to proceed are based on scientific merit, peer approval and clinical feasibility.

- **Incentives for Participation and Tailored Funding** The funding mechanism requires investigators to apply annually for peer-reviewed set-aside funds (set-aside funds are restricted funds within each investigator's grant). For set-aside funds to be released, the investigator must demonstrate collaboration with other EDRN investigators. Participation in steering committee meetings are an explicit requirement of the grant and the group is small enough so that teams can work effectively together. Communal investment is critical and promoted. One of the Network's strengths, the structure of the grant mechanism created culture change whereby the group succeeds together.

- **Core Services for Early Translational Research** EDRN published metrics on the five phases of biomarker development. Dialogue about how to successfully move biomarker discovery projects forward into validation is a constant. The Enterprise Research Network Exchange (ERNE) monitors all tissue and specimen resources in EDRN. The Network addressed barriers in core services. Sometimes it is difficult, using the consensus process, to have finite position statements on the best ways to collect specimens. EDRN advocates establishing consensus standards and developed standard operating procedures (SOP) for providing a ready source of serum or plasma for proteomic, methylation and other studies. Cooperative groups, clinically focused investigators and basic scientists benefit.

- **Management Structure for Early Translational Research** EDRN has an explicit structure to identify, facilitate and coordinate access to resources and collaborators. EDRN requires investigators to attend and participate at meetings (three per year) as a condition of funding. Set-aside monies are not available to investigators unless they demonstrate collaborative efforts. Integration with other NCI-funded activities is actively supported and encouraged. ■

CHAPTER TEN

Investing in Biomarker Research for Early Detection

"To achieve our vision of modern medicine, we also need research scientists with broad expertise, from widely varied disciplines, coming together in highly cooperative and efficient teams to answer ever more complex questions. To this end, NIH recently changed a long-held policy of having only a single principal investigator on any NIH grant to a new policy that allows, when appropriate to the science, multiple principal researchers to apply for a grant together. This new policy is encouraging collaboration across disciplines and enabling academic scientists to exercise creative leadership in a project while bringing more of the best and brightest from physical, biological and behavioral sciences to the task of solving the multifaceted and complex health-related problems."

Elias A. Zerhouni, M.D., Director
National Institutes of Health, March 6, 2007

AS PART OF AN INITIATIVE to evaluate NCI's effort in **translational research**, the NCI Translational Research Working Group (TRWG) analyzed the fiscal year 2004 research portfolio. For the first time, NCI projects funded through a variety of mechanisms that fit the definition of research that "transforms scientific discoveries arising from laboratory, clinical, or population studies into clinical applications to reduce cancer incidence, morbidity and mortality" were assessed.

TRWG constructed six "developmental pathways" that characterize the transformation of scientific discoveries into new clinical modalities for oncology. These modalities are categorized as:

1. **Risk assessment modalities,** intended to characterize the cancer-related health status of an individual, which includes biospecimen-based risk assessment devices (protocols, reagents, instruments), image-based risk assessment (agents or techniques); and
2. **Interventive modalities,** intended to change the cancer-related health status of an individual via prevention or treatment, which includes agents (drugs or biologics), immune response modifiers (vaccines, cytokines, etc.), interventive devices and lifestyle alterations.

The developmental pathway diagrams, such as the one shown in Figure 10-1, specify key activities and decision points, clarify dependencies among corresponding steps and events and show important feedback loops and iterative processes that are embedded within the development process.

In many ways, the initiatives set forth by TRWG parallel the existing management structure of EDRN (see Chapter 9). Steps similar to those in the pathway schema have already been implemented by the Network, allowing EDRN to effectively achieve its goals despite some limitations related to scope, funding and staffing constraints.

TRWG found that approximately $1.33 billion of the total $4.4 billion NCI fiscal year 2004 funding was awarded for translational research projects (see Figure 10-2). About 41% ($547 million) of that was dedicated to program and cooperative awards. Of those awards, 4% ($21.8 million) was awarded to EDRN.

NCI Investment in Translational Research

EDRN was envisioned as an approach to provide a seamless translational research pathway that would connect a diverse group of outstanding investigators into a productive unit capable of sharing ideas, technologies, skills and products. Ample financial support and incentives for collaboration were an indispensable aspect of the program. NCI sought to create the critical mass necessary to invent and validate new tools and to exploit novel technologies to enhance cancer screening and early detection and ultimately reduce cancer mortality.

Other NCI programs have been established to realize clinical use of diagnostic **biomarkers** (see Figure 10-3). Future infrastructure improvements rely on continuing improvements in the Network's collaborative culture, which the EDRN established despite an already entrenched culture that rewarded individual achievement rather than collaborative work, through the funding process.

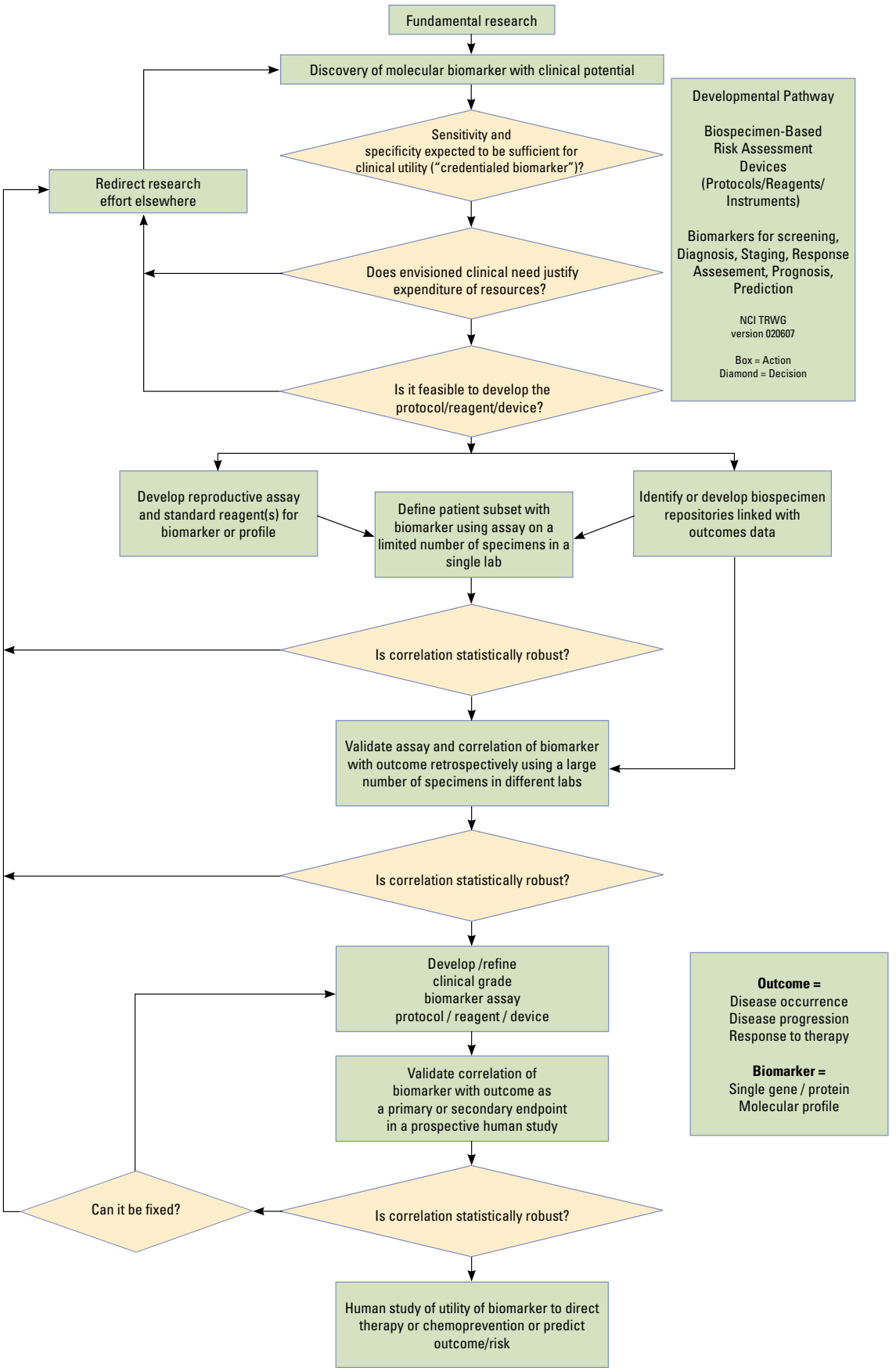
As discussed in Chapter 9, EDRN produced hundreds of publications and well over 100 biomarkers in the discovery phase. The cost-effectiveness of the biomarkers effort could only be realized after there are a number of fully validated and clinically useful biomarkers in healthcare settings. This, however, will require a sustained, long-term investment.

EDRN's portfolio of biomarkers is expanding and its collection of sample sets, critically important for both discovery and **validation,** is growing. Over the next 2 years, at least one and likely more biomarkers for early detection of common, high-mortality cancers (colon, breast, lung or prostate) will enter Phase 2 or 3 validation trials. Continued investment in strong analytical technology, informatics, statistics, epidemiology and biosample management will pay dividends through high-quality data that will meet regulatory requirements.

An analysis was performed to evaluate NCI biomarker grants in the area of early detection and diagnosis and to analyze trends in publications, patents and collaborations that resulted from these grants. Specifically, biomarker-related grants initially funded in FY1999 or FY2000 by the EDRN (a U01 mechanism) or by other grant mechanisms (R01 and other U01 programs) were tracked through FY2005 and the metrics of their success (patents, publications and collaborations) were evaluated.

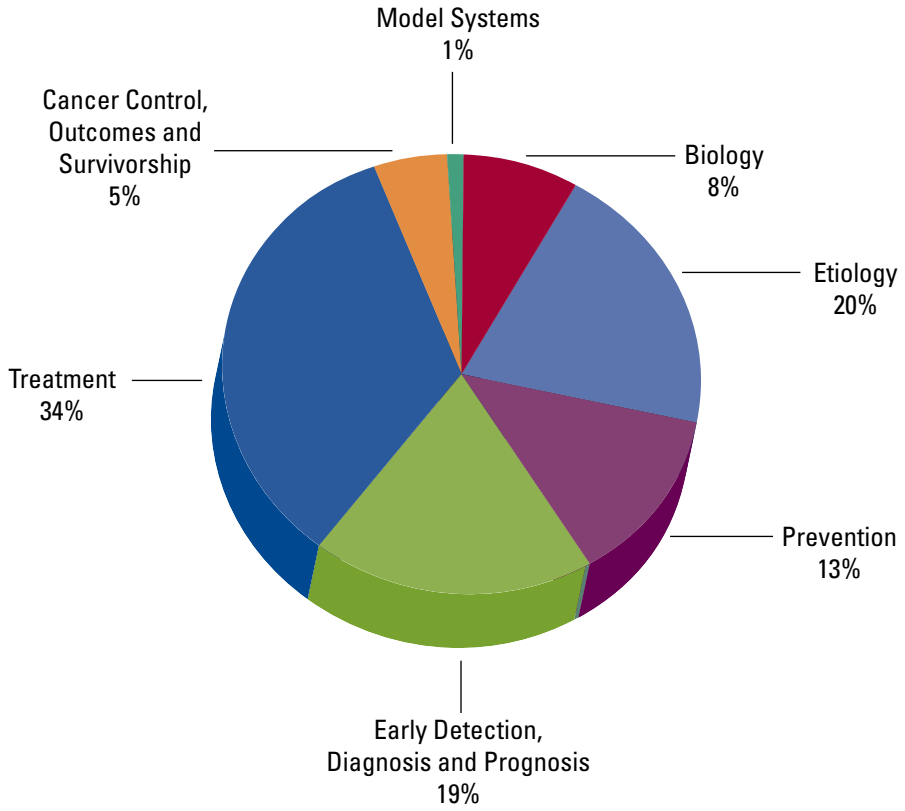
The average number of cumulative publications through 6 years post-grant initiation per award type is shown in Figure 10-4. EDRN projects (U01) yielded an average of 14 publications per grant, non-EDRN U01s yielded an average of 11 publications per grant and R01s yielded an average of five publications per grant. The average number of publications per million dollars invested by NCI is shown in Figure 10-5 for each award type. R01 biomarker grants yielded an average of 2.8 publications per million dollars invested, non-EDRN U01s yielded an average of 2.9 publications per million dollars and EDRN grants yielded an average of 3.2 publications per million dollars.

Figure 10-1. Translational Research Developmental Pathway



Source: *http://www.cancer.gov/images/trwg/Biospecimen-RiskAssessmentPathway050807.pdf*

Figure 10-2. NCI Investment in Translational Research



Source: *http://www.cancer.gov/trwg/portfolio-analysis.pdf*

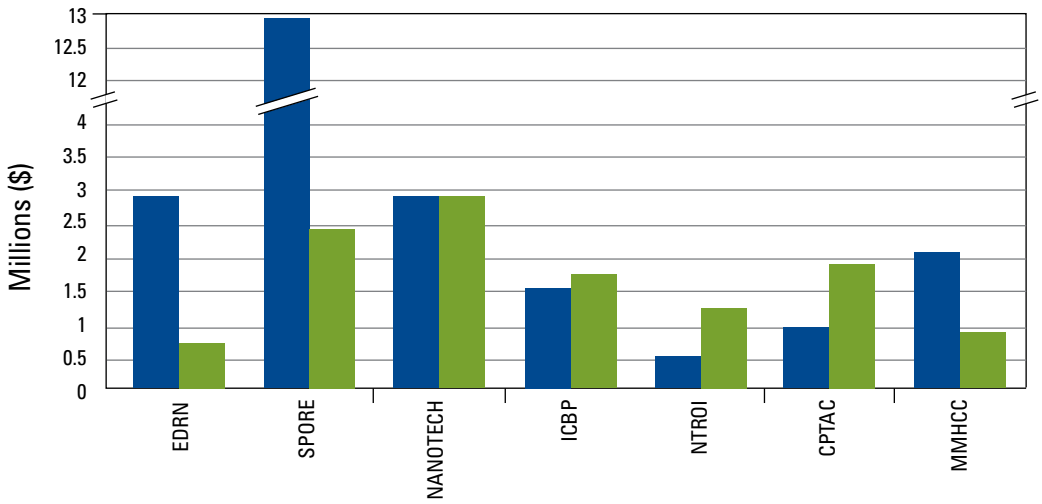
As shown in Figure 10-6, the articles resulting from these NCI-funded biomarker grants (R01, non-EDRN U01 and EDRN U01) had an average impact factor of six. The majority (75%) of these articles were published in journals with an impact factor of 3 to 20, while a few articles were published in journals with an impact factor greater than 20 (see Figure 10-7). Impact factor measures the number of citations to science journals.

EDRN investigators generated more than 26 patents and 14 licenses with diagnostic companies willing to invest in EDRN-developed biomarkers for further development and eventual submission to FDA. These outcomes are likely to improve over the next 5 years, since a significant portion of EDRN efforts during the first 5 years was directed toward the organization, coordination and management.

Challenges and Solutions

The EDRN collaborative group infrastructure has matured, with the greatest achievements occurring during the last 3 years. Individual groups have built differing approaches to promote and support collaborative research, but each developed a portfolio of products and technologies that can exploit the translational research resources of the group.

Figure 10-3. Major NCI-Funded Translational Research Programs, FY 2006



EDRN – Early Detection Research Network
SPORE – Specialized Programs of Research Excellence
NANOTECH – Nanotechnology Program
ICBP – Interactive Cancer Biology Program
NTROI – Network for Translational Research in Optical Imaging
CPTAC – Clinical Proteomic Technology Assessment for Cancer
MMHCC – Mouse Models of Human Cancer Consortium

Source: Adapted from the NCI Translational Research Working Group

For example, the Prostate and Urologic Cancer Collaborative Group developed microsatellite markers for bladder cancer and SELDI/MALDI-TOF for prostate cancer. The Colorectal and Other Gastrointestinal Cancers Collaborative Group developed and implemented a validation trial of des-carboxyprothrombin (DCP) for the detection of hepatocellular carcinoma. The sample set collected for this validation trial is being leveraged for use as prevalidation samples (Phases 1 and 2). EDRN Biomarker Developmental Laboratories (BDLs) have studied new markers (e.g., GP73, a novel **glycoprotein** marker of liver disease) and technologies (such as proteomics profiles) using a subset of this sample set. The derived data will determine whether these additional biomarkers for the early detection of hepatocellular carcinoma should be validated in a Phase 2 cross-sectional trial. Similarly, the Lung Collaborative Group developed a panel of **epigenetic** biomarkers that are being studied with a reference set that was jointly collected by members of the group.

Through the funding of new members and investment in Associate Memberships, EDRN's technological infrastructure markedly improved. Improvements have raised the quality and reproducibility of complex technologies such as proteomics, antibody arrays and **genomics** tools. For instance, Ambergen, Inc. brought a new, automated artificial gene expression system to EDRN that solves many of the problems of heterogeneity in detecting multiple mutations at a given genetic locus. An EDRN Clinical Epidemiology and Validation Center (CEVC) and an EDRN BDL are collaborating with Ambergen to detect common colorectal adenocarcinoma associated mutations in the *APC* gene in DNA extracted from human urine. The new technology captures 85% of

Figure 10-4 to 10-7. Trends in Early Detection and Diagnosis Grants

Figure 10-4. Average Number of Publications per NCI-funded Biomarker Grant, FYs 2000-2005

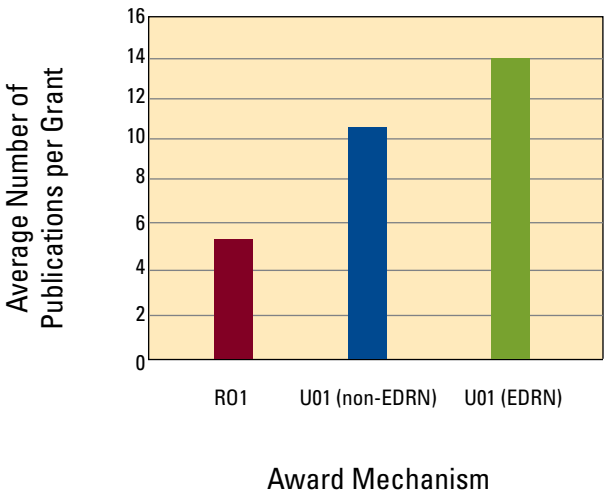


Figure 10-5. Average Number of Publications per Million Dollars Invested for NCI-related Biomarker Grants that Began in FY1999 or FY2000 to 2005

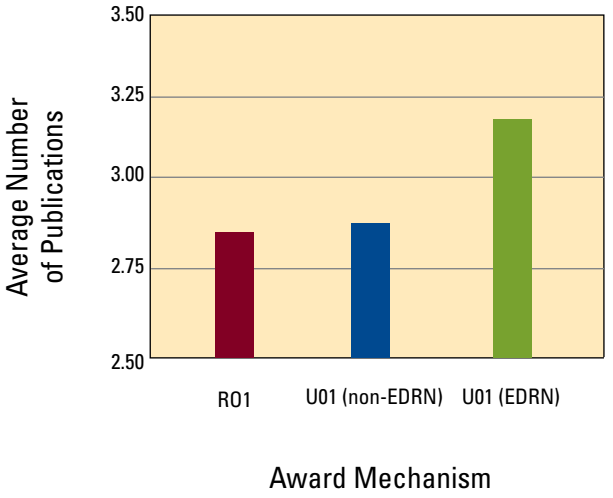


Figure 10-6. Average Impact Factors for Publications Resulting from NCI Biomarker Grants that Began in FY1999 or FY2000 to 2005

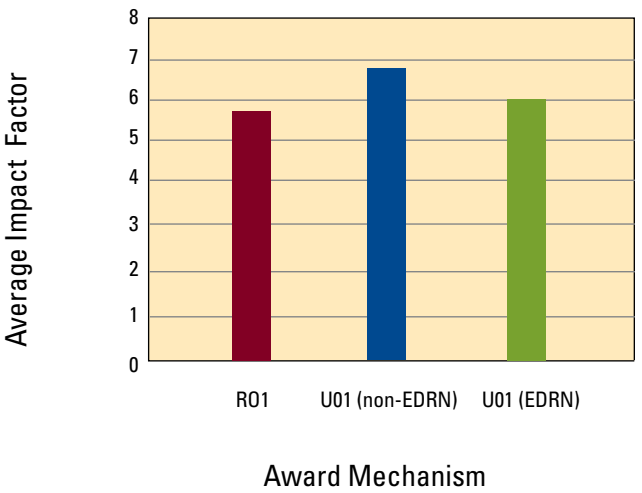
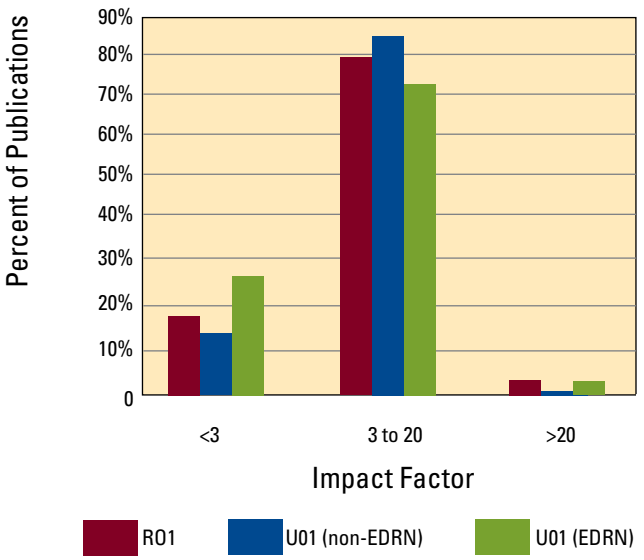


Figure 10-7. Impact Factors for Publications Resulting from NCI Biomarker Grants that Began in FY1999 or FY2000 to 2005



Source: Analysis done by SAIC-NCI

all known *APC* mutations in a single, high-**throughput**, automated assay and creates a new, potentially more efficient and cost effective early detection and cancer screening tool.

The progression of biomarkers from the discovery phase to the validation phase is slow to date, reflecting initial challenges with cultural and infrastructural issues. Perhaps the most important challenge facing EDRN has been to create and nourish a culture of collaboration that attracts top-level scientists, most of whom have been accustomed to working in and being rewarded for less collaborative work.

At the EDRN's inception, the diversity of scientific backgrounds caused immediate clashes due to problems in communication, misplaced incentives and individualistic tendencies to work separately. Numerous conflicts have since been resolved through a high-quality, respected internal peer-review process that sets the tone for the future. At the same time, NCI program staff worked fairly, systematically and strategically to defuse discord that could shut down group-to-group teamwork.

There is a growing consensus of collaboration and credit-sharing within and between the EDRN organ-based collaborative groups. Substantial attention is paid to grappling with problems that previously were considered simplistic; an example is the quality control of sample collection and management. EDRN investigators invest considerable effort and time into building excellence in these critical resources.

Some investigators fail to recognize the importance of sharing resources and expertise and continue to see EDRN mainly as a source of funding for their laboratory-based research, not as a place where the products of this research can be brought for validation and generalization. Most EDRN investigators, however, choose to work through the Network precisely because of its translational research vision, which allows clinical, epidemiology and statistical research groups' access to investigators and technologies that would otherwise not be available. Many investigators have established strong collaborative ties with first-rate laboratories across the country and internationally that have enabled implementation of the translational research paradigm.

When EDRN was initiated, the translational infrastructure necessary to meet the premise of the Network was non-existent. For example, there were no quality control mechanisms to ensure reproducible laboratory analytical performance, no common data elements (CDEs) required to work with human biosamples, no quality human biosample collections with appropriate good clinical practices and good computing practices. Informatics needed development to manage large amounts of clinical and biological data. Problems with the increasing burden of regulation—human subjects protections, materials transfer agreements and intellectual property protections—caused delays and problems in information exchange and in the willingness of investigators to collaborate.

By leveraging other government resources, such as the National Aeronautics and Space Administration's (NASA) Jet Propulsion Laboratory, EDRN developed a novel informatics infrastructure (see Chapter 7) that permits interrogation of diverse databases at long distances. This infrastructure enhances group cohesion and provides investigators with the critical information about biosample quality and quantity that permits development of collaborative translational projects. EDRN built a set of CDEs for translational research that included demographic, clinical, biosample, research and clinical data. Development and utilization of these data elements have been critical factors in creating group cohesion and in linking resources from diverse units throughout EDRN.

EDRN learned from these problems, grown scientifically and culturally and transmitted lessons learned to other parts of the NCI and the wider scientific community. Obstacles, should not be interpreted as weakness of the concept or the model rather, they should be interpreted as a process of evaluation and change as the commitment of the stake-holders and the scientific community alike continues to grow collectively.

The first grant solicitations to establish EDRN specified multiple units to create a cohesive translational paradigm for the discovery and validation of biomarkers for cancer early detection and risk assessment. As noted, early performance was uneven. CEVCs, in collaboration with others components of EDRN, have introduced new products and tools for development in EDRN for prostate, lung, bladder, esophageal, hepatocellular, pancreatic and colorectal adenocarcinomas. Examples in the GI and Lung Collaborative environment include the detection of epigenetic changes in **promoter** regions of tumor suppressor genes; the ability to rapidly screen exons of tumor suppressor genes in human stool, urine and plasma; new-generation proteomics tools that rapidly detect and identify differentially over- and underexpressed proteins in prostate, lung, colon and liver diseases; and, automated FISH-based technology for the detection of neoplasia associated mutations.

EDRN 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. The data collection and sample collection processes and analytic procedures are continuously reviewed and audited by the Data Management and Coordination Center (DMCC). The data developed and disseminated by EDRN is expected to be of high quality and the multi-site EDRN validation trials are likely to substantiate the prevalidation data. Hence, the high quality of the data, the produced documentation and multi-site infrastructure will permit such data to be used for regulatory review and approval.

The Associate Membership Program was highly productive in bringing new technologies and products into the Network. More than 120 Associate Members from academia and industry have joined EDRN.

Practical challenges have also been faced, including Intellectual Property (IP) issues posed by a number of patented and licensed tools and technologies. Frequent questions are: Who owns the IP for collaborative products resulting from individually licensed reagents and assays; and how would the IP of an individual investigator be protected? EDRN developed IP guidelines and requires investigators to share their IP plans.

Collaborative projects often involve transfer of specimens from one institution to another, requiring separate Material Transfer Agreements (MTA) between the providers and the receiving institutions. Despite the guidance provided by NIH, each institution has its own MTA requirements. Such constraints continue to hamper completion of collaborative projects in a timely manner, sometimes resulting in the loss of interest and motivation among participating members.

EDRN's Impact on Cancer Prevention Research

Without EDRN, research into new biomarkers of early cancer detection and risk would have remained on the periphery of research with a strong, but fragmented laboratory presence and little translational interest in the academic scientific community. But with the Network, a new translational paradigm is defining the organization, approaches and standards by which biomarkers are developed and assessed. The Network created major focus, energy and new research in the field of early detection. The Network's publications, meetings, funding opportunities and infrastructure have fashioned a new environment for cancer prevention research.

EDRN's work represents a paradigm shift that brought international attention, new investigators and increasing involvement by academic and industry communities. For example, two companies with major markets in diagnostics, Abbott Molecular, Inc. and Roche, Inc.; by collaborating with Network scientists promise a strong

marketing pathway for EDRN-discovered products. Another impressive indication is the increase of solicitations for NCI-industry-focused meetings on biomarker technology, development, validation or regulation.

An estimated total cumulative NCI investment per American over the past 30 years is about $258, or about $9 per American per year over the entire period, NIH Director Elias Zerhouni, M.D., told Congress in 2007. He cited EDRN as one of the major programs with significant outcomes for the investment. Because of a hundredfold reduction in the unit cost of **genomic** technology, researchers can now study at affordable costs, he noted.

EDRN's approach fits with the NIH's research paradigm for the future, which seeks to transform medicine from curative and reactionary to preemptive and anticipatory. As Dr. Zerhouni testified, "A more **predictive**, personalized and preemptive form of medicine is no longer just a dream but a vision to strive for, because it can reduce disease burden and its costs while improving individual quality of life." Other NIH institutes have emulated the EDRN model for their respective clinical programs, such as Rare Disease Research Network (RDRN), Office of Director, NIH; Network for Translational Research in Optical Imaging (NTROI), NCI; and the Osteoarthritis Initiative, National Institute of Arthritis, Musculoskeletal and Skin Diseases, NIH.

The pipeline of biomarkers to be studied in a prevalidation environment appears to be growing. The funnel analogy suggests that the large bulk of biomarkers under study in EDRN will be in discovery and prevalidation stages. Within the next two years, it is expected that at least three validation studies will be completed on bladder, hepatic, lung and prostate cancers. Biomarkers or biomarker panels from the GI area—a serum-based test using nuclear-matrix proteins for the detection of colorectal adenocarcinoma, a stool- and urine-based gene panel of four genes for the early detection of colorectal cancer and a 4-gene FISH-based panel for detection of adenomatous neoplasia of the lower esophagus—derived from EDRN collaborative research will be entering Phase 2 (cross-sectional validation).

By the end of the current grant period (2010), it is expected that at least one and probably two to three, biomarker products will have been submitted to the FDA for regulatory approval. The des-carboxyprothrombin validation trial for the early detection of hepatocellular carcinoma and microsatellite markers trial for bladder cancer may generate a sufficient quality and quantity of data to justify FDA review for approval as early diagnostic products.

Thus, the Network's structure provides a solid approach to early translational research. Discovery leads to work that confirms and improves the accuracy of the biomarker, which then moves to early clinical validation of the test. Through this approach to early translational research EDRN built and implemented a vertically integrated pipeline of biomarkers for cancer early detection and risk assessment. The Network attracts excellent academic and industry scientists by providing access to diverse top-quality assays, clinical specimens, methodological expertise, industrial resources and financial resources that are not organized or readily available through other governmental or industry funding mechanisms.

This Network structure within the NCI vision of early translational research is expected to lead in a few short years to the molecular diagnostics that will allow physicians and health care professionals to prevent or eliminate many cancers and ultimately transform cancer into manageable, rather than fatal, diseases. ■

APPENDIX I

Key Publications by Investigators in the Early Detection Research Network

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APPENDIX II

Publications Co-Authored by NCI Program Staff

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GLOSSARY

The entries defined here are highlighted in bold type at the first occurrence in each section of this report.

Biomarker – A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic response to a therapeutic intervention.

Bioinformatics – Computational analysis and management of biomedical information.

Clinical endpoint – A characteristic or variable that reflects how a patient feels, functions or survives.

Clonal changes – Changes observed in a subset of a larger group of cells originating from a single parent cell.

Enzyme-linked immunosorbent assay (ELISA) – A method where antibodies are used to quantify levels of a biological marker.

Epigenetics, epigenomics – The study of events affecting the functional state of DNA and gene expression without changing its sequence or linear arrangement.

Fluorescence in situ hybridization (FISH) – A technique using fluorescent probes to visualize locations of specific gene sequences on chromosomes. Often used for gene mapping and identifying chromosomal abnormalities.

Fucosylation – The process of attaching a fucose sugar unit to a molecule.

Genomics – Characterization of the entire DNA and gene expression within a cell, tissue, or organism.

Glycomics – The study of the structure and function of all complex carbohydrate structures from a biological source.

Glycoprotein – A protein with attached sugar structures.

Haplotype – An assortment of DNA sequence or gene variations that are typically coinherited as a unit on a single chromosome.

Horizontal approach – Characterized by a number of independent players or entities in an organization, generating economies of scales embedded with duplicated efforts and coordination challenge

Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) – A platform for profiling a population of proteins by mass spectrometery according to the size and net charge of individual proteins. The peaks identified by this method require further analysis to determine the identity of the corresponding proteins.

Methylation – The addition of a methyl group to specific sites on DNA. The methylation of a gene can change its expression.

Methylation specific PCR (MSP) – An adaptation of PCR to identify and quantitate relative levels of methylated genes in DNA.

Microarray – A system of printing large numbers of DNA sequences, proteins, antibodies or tissue lysates on a slide which can then be analyzed in a high-throughput fashion.

Microsatellite (Instability) Analysis (MSA) – Microsatellites are short sequences of DNA, usually 1 to 4 base pairs in length, repeated any number of times in various locations of DNA. Microsatellite instability analysis is a test to determine if the number of repeating units has changed at any specific location(s).

Ontology model – A way to describe critical cancer data objects. An ontology model is a conceptual model used to represent knowledge in a domain (e.g., management of biomarkers).

Polymerase chain reaction (PCR) – A technique to amplify or produce multiple copies of a defined DNA span.

Predictive value [positive/negative] – The fraction of people who test [positive/negative] and [have/do not have] the disease.

Promoter – The segment of a gene where expression is regulated by binding specific proteins to initiate mRNA transcription.

Proteomics – Characterization of all proteins from a biological source.

Quantitative PCR (qPCR) / quantitative Methylation Specific PCR (qMSP) – An adaptation of PCR to quantify levels of defined mRNA transcripts or methylated genes.

Risk stratification (prediction) – Quantifying the relative level of risk for a disease based on defined criteria.

Sensitivity – The proportion of individuals with a disease who test positive.

Specificity – The proportion of individuals without a disease who test negative.

Standard specimen reference sets (SSRSs) – collections of high quality, well-characterized specimens that can be used for discovery and early validation of potential markers.

Surface-enhanced Laser Desorption-Time of Flight (SELDI-TOF) – A modification of MALDI-TOF where some selectivity of proteins can be achieved prior to analysis.

Throughput – The number of samples that can be processed in a defined time period.

Translational research – Studies intended to bring developments from laboratory investigation to clinical application.

Validation – Confirmation of the accuracy, precision, or effectiveness of experimental results.

Vertical approach – Characterized by distinct job classifications or responsibilities among entities and vertically-defined flow of decision-making free from the burden of coordination with other entities.