National Cancer Institute • **Division of Cancer Prevention**

The Early Detection Research Network

Translational Research to Identify Early Cancer and Cancer Risk

Second Report • October 2002

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Foreword

We are in the midst of a biomedical revolution, where multidisciplinary collaboration will translate the breakthroughs of basic research swiftly from the laboratory to the clinic. Discovery of the molecular signatures of cancer will make possible powerful, new tools for detecting cancer and cancer risk–this is the focus of the National Cancer Institute's Early Detection Research Network.

The Network has a straightforward mission: to translate newly emerging molecular knowledge into practical clinical tests to detect cancer and cancer risk. For most cancers, successful treatment depends on early detection and successful prevention depends on the accurate evaluation of risk. The Early Detection Research Network seeks to give treatments the opportunity to work and to make prevention possible.

The Network is using cutting-edge technologies to identify the changes that occur in the earliest stages of a cell's transformation onto the road of cancer. Scientific expertise from leading national and international institutions has been harnessed to first identify, and then validate, crucial molecular markers to detect cancer and to assess cancer risk. The Network is an investigator-initiated consortium for the collaborative research to link the discovery of biologic markers directly to the next steps in the process of developing early detection tests.

I am pleased to introduce this Second Report on the Early Detection Research Network, a flagship program of the National Cancer Institute's Division of Cancer Prevention. In the two years since the Initial Report, the Network has made critical progress, discovering many promising biomarkers and moving several into crucial validation studies. The power of bioinformatics and computer-assisted programs are being put to full use to analyze Network data and to facilitate faster answers to key questions. Collaborations and partnerships that are necessary for our ultimate success have been put into place.

The Network is an opportunity and a challenge for the scientific community – an opportunity to make science work for people and a challenge to make this new-found model of collaboration a productive scientific construct. This type of translational research is our first step toward transforming cancer medicine and improving public health in the 21st century.

Andrew C. von Eschenbach, M.D.
Director
National Cancer Institute

Introduction

He is a better physician that keeps diseases off us, than he that cures them being on us; prevention is so much better than healing because it saves the labour of being sick.

THOMAS ADAMS, 1618

Detecting cancer in its initial stages presents the opportunity to treat disease before it spreads. Further, the ability to reduce a person's risk of developing cancer opens the way for optimum prevention strategies. The National Cancer Institute (NCI) is committed to progress in cancer detection and risk assessment that allows interventions to focus on the earliest stages of disease.

The burden of cancer has been significantly reduced since enactment of the 1971 National Cancer Act, but sustained research and clinical efforts are necessary to continue the trend. While cancer incidence and mortality rates declined overall by about 1.1 percent per year from 1992-1998, cancer remains a leading cause of death in the United States.

The Early Detection Research Network is a scientific consortium funded and coordinated by NCI's Division of Cancer Prevention. In its third year, the Network is the nation's premier program for systematic identification, development, and validation of novel biological markers, known as biomarkers, which distinguish the characteristics of cancer cells. The Network has produced a system for evaluating biomarkers as tools to clinically detect cancer before symptoms appear, and to identify people at risk.

The Network's Initial Report, published in September 2000, described the strategic design that enables researchers across disciplines and institutions to develop and validate biomarkers as a team within a streamlined process. Guiding principles for the forward movement of biomarkers from the laboratory to clinical testing were also detailed.

The highlights described in this Second Report focus on the progress made by scientists within the Network's research infrastructure; the rigorous work needed to prove biomarkers can strengthen health outcomes; and the research milestones of novel biomarkers currently in development or in validation.

NCI's Division of Cancer Prevention has a core mission to conduct and support research to improve the health of the public by decreasing the incidence, mortality, and morbidity of cancer. The division is the primary NCI unit devoted to cancer prevention research and is directed by Peter Greenwald, M.D., Dr.P.H.

More specifically, the Division of Cancer Prevention:

- Plans and directs an extramural program of cancer prevention research, including chemoprevention, nutritional science, genetic and infectious agents, biometry, and early detection including biomarker development and validation;
- Develops and supports research training and career development in cancer prevention and early detection;
- Coordinates program activities with other Divisions, Institutes, or federal and state agencies, and establishes liaison with professional and voluntary health agencies, cancer centers, labor organizations, cancer organizations, health care delivery and managed-care organizations, and trade associations; and

- Coordinates community-based clinical research in cancer prevention, and dissemination of cancer treatment practices through a consortium of community clinical centers.

Within the Division of Cancer Prevention, the mission of the Cancer Biomarkers Research Group is to engage basic and clinical scientists as well as epidemiologists and statisticians in a search for and validation of promising early cancer biomarkers. To do so, the Cancer Biomarkers Research Group supports and facilitates a broad spectrum of national and international research activities in molecular biology and genetics, particularly for the discovery of biomarkers for risk prediction and early detection.

The Cancer Biomarkers Research Group also supports the development of databases and informatics systems to optimize tracking and assessment of biomarker utility and expression patterns. By facilitating, promoting, and coordinating cutting edge research with the latest discoveries in technology and molecular circuitry of preneoplastic cells, the Cancer Biomarkers Research Group hopes to provide a mechanistic picture of preneoplastic progression and tools for effective cancer prevention and clinical management. The mission will be accomplished through a mix of program portfolios, including grants, contracts, and program-initiated research.

Sudhir Srivastava, Ph.D., M.P.H., is chief of the Cancer Biomarkers Research Group and program director for the Early Detection Research Network.

Timeline

November 13, 1998	Early Detection Implementation Group proposes concept for Early Detection Research Network to NCI Board of Scientific Advisors. Concept is approved.
October 6, 1999	Eighteen Biomarkers Developmental Laboratories' funds awarded.
April 2000	Early Detection Research Network fully launched and First Steering Committee meeting is held in St. Petersburg, Fla.
May 2000	Funds awarded for the three Biomarkers Validation Laboratories, nine Clinical and Epidemiological Centers, and the Data Management and Coordinating Center.
June 2000	Task Force for molecular taxonomy of preneoplasia convened.
September 2000	First round of proposals for collaborative studies approved.
September 25-27, 2000	Second Steering Committee meeting and first Scientific Conference held in Chicago, Ill.
October 2000	Initial Report of Early Detection Research Network published.
January 21-22, 2001	Third Steering Committee meeting convened in San Antonio, Texas.
May 22, 2001	Progress Report presented to the National Cancer Advisory Board.
June 22-23, 2001	Fourth Steering Committee and the First Network Consulting Committee meetings convened in Washington, DC.
June 26, 2001	Progress Report presented to the Board of Scientific Advisors.
October 13-15, 2001	Second EDRN Workshop convened in Seattle, WA.
February 3-5, 2002	Fifth Steering Committee and Second Network Advisory Committee meetings convened in Houston, Texas.
March 10-15, 2002	Gordon Research Conference on New Frontiers in Cancer Detection and Diagnosis, held in Ventura, CA.
September 3-6, 2002	Sixth Steering Committee Meeting convened in Ann Arbor, MI.
October 3-4, 2002	Third Network Consulting Committee Meeting, Washington, DC.
November 14-15, 2002	Progress Report presented to NCI Board of Scientific Advisers.
January 29-31, 2003	Seventh Steering Committee meeting, held in Birmingham, AL.

Components of the Early Detection Research Network

The Early Detection Research Network was conceived on the premise that a "vertical" approach to biomarker research—that is, an established integrated, multidisciplinary environment—will facilitate collaboration among technology developers, basic scientists, clinicians, epidemiologists, biostatisticians, and other health professionals, and therefore expedite clinical applications of the molecular knowledge that has burgeoned in recent years.

Biomarker research occurs in phases: initial discovery of biological markers, evaluation of the most promising biomarkers, and validation to determine that they can work in a clinical setting. The consortium of collaborating investigators is dedicated to working in a systematic and concerted fashion to speed translations of basic scientific discoveries in genomics and proteomics into medical benefits.

Structured around four main components, the Network comprises a group of 18 Biomarkers Developmental Laboratories, three Biomarkers Validation Laboratories, nine Clinical and Epidemiology Centers, and a single Data Management and Coordinating Center.

- *Biomarkers Developmental Laboratories* develop and characterize new biomarkers and cancer signatures.
- *Biomarkers Validation Laboratories* serve as a resource for clinical and laboratory validation of biomarkers, including technological development, standardization of assay methods, and refinement.
- *Clinical/Epidemiology Centers* conduct the early phases of clinical and epidemiological research on the application of biomarkers.
- *Data Management and Coordinating Center* provides statistical, logistics and informatics support, and develops the theoretical statistical approaches to pattern analysis of multiple markers simultaneously.

A Steering Committee, comprised of the Network's Principal Investigators and NCI staff, coordinates the work of the consortium and provides major scientific management oversight. The group is responsible for developing and implementing protocols, designs, and operations. An Executive Committee of the Steering Committee meets monthly; it is comprised of chairs for the Collaborative Groups, the NCI program director, the Steering Committee chair and co-chair. Five subcommittees and one working group report to the full committee.

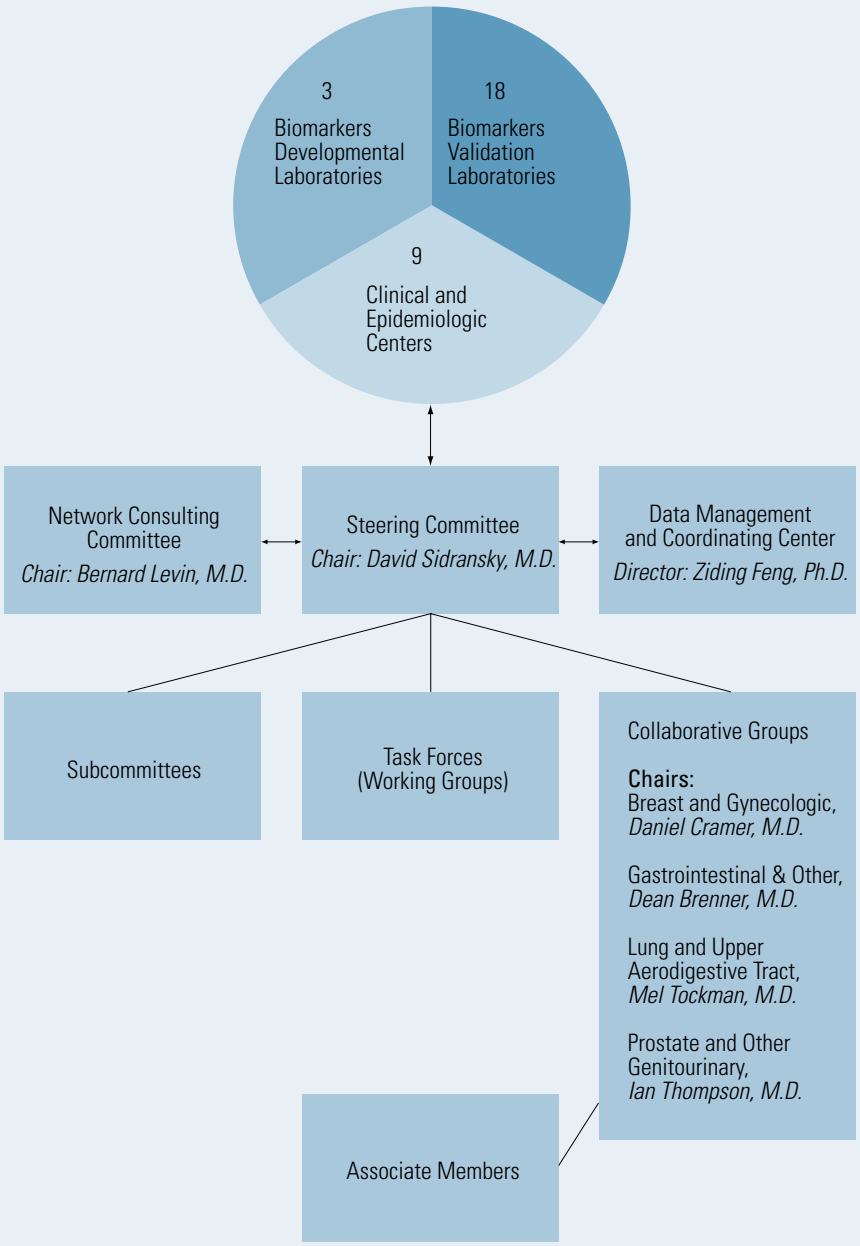
Additional collaborations are encouraged through the Associate Membership program, which supports one-time pilot projects, ongoing resource sharing projects, and open participation in meetings, workshops, and conferences by non-Network professionals with a strong interest in early detection of cancer.

Within the Network structure, Collaborative Groups, open to any investigator, focus on specific organ sites to exchange information and facilitate research on organ-related biomarkers. Network Principal Investigators are elected by group members to serve as chairs. Chairs frequently invite outside experts to talk about research related to potential collaborations. The Groups meet in conjunction with the Steering Committee meeting to discuss and prioritize the biomarker research agenda for their respective organ sites:

breast and gynecologic cancers; G.I. and other associated cancers; lung and upper aerodigestive cancers; and prostate and urologic cancers. Chairs report on the deliberations to the Steering Committee.

Members of the Consulting Committee are not investigators in the Early Detection Research Network. Their role is to review the Network's progress, recommend new research initiatives, and ensure the Network is responsive to promising opportunities.

Components of the Early Detection Research Network



Principal Investigators

Clinical and Epidemiologic Centers

Principal Investigator	Institute	Organ Focus
Elizabeth R. Unger, M.D., Ph.D.	Centers for Disease Control and Prevention, Atlanta, GA	Cervix
Kathy Helzlsouer, M.D.	Johns Hopkins University, Baltimore, MD	Breast
Alan Partin, M.D., Ph.D.	Johns Hopkins University, Baltimore, MD	Prostate
Daniel W. Cramer, M.D.	Brigham and Women's Hospital, Boston, MA	Ovary
Dean E. Brenner, M.D.	University of Michigan, Ann Arbor, MI	Colon
Henry T. Lynch, M.D.	Creighton University, Omaha, NE	Pancreas
William N. Rom, M.D.	New York University School of Medicine, New York, NY	Lung
Margaret R. Spitz, M.D., M.P.H.	University of Texas, M.D. Anderson, Houston, TX	Head & Neck
Ian M. Thompson, M.D.	University of Texas Health Science Center, San Antonio, TX	Prostate

Biomarkers Developmental Laboratories

Principal Investigator	Institute	Organ Focus
Wilbur Franklin, M.D.	University of Colorado Health Science Center, Denver, CO	Lung
Jose Costa, M.D.	Yale University, New Haven, CT	Breast, Colon, Pancreas
Melvin Tockman, M.D., Ph.D.	University of South Florida, Tampa, FL	Lung
David Fishman, M.D.	Northwestern University, Evanston, IL	Ovary
David Sidransky, M.D.	Johns Hopkins University, Baltimore, MD	Lung
Bruce Trock, Ph.D.	Johns Hopkins University, Baltimore, MD	Breast
Edward Highsmith, Jr., Ph.D.	University of Maryland, Baltimore, MD	Variety of sites
Stephen Meltzer, M.D.	University of Maryland, Baltimore, MD	Esophagus

David Beach, Ph.D.	Genetica, Inc., Cambridge, MA	Breast, Colon
Samir Hanash, M.D., Ph.D.	University of Michigan, Ann Arbor, MI	Variety of sites
Jeffery Marks, Ph.D.	Duke Medical Center, Durham, NC	Breast
Timothy Block, Ph.D.	Thomas Jefferson University, Doylestown, PA	Liver
William L. Bigbee, Ph.D.	University of Pittsburgh, Pittsburgh, PA	Bladder, Colon, Lung
Adi Gazdar, M.D.	University of Texas, Southwestern Medical Center, Dallas, TX	Lung
Yingming Zhao, Ph.D.	University of Texas, Southwestern Medical Center, Dallas, TX	Ovary, Prostate
Bogdan Czeriniak, M.D., Ph.D.	University of Texas, M.D. Anderson Cancer Center, Houston, TX	Bladder
John O. Semmes, Ph.D.	Eastern Virginia Medical School, Norfolk, VA	Prostate
Nancy Kiviat, M.D.	University of Washington, Seattle, WA	Ovary, Prostate

Biomarkers Validation Laboratories

Principal Investigator	Institute	Organ Focus
William E. Grizzle, M.D., Ph.D.	University of Alabama, Birmingham, AL	All sites
David Chia, Ph.D.	University of California, Los Angeles, CA	All sites
Peter E. Barker, Ph.D.	National Institute of Standards and Technology, Gaithersburg, MD	All sites

Data Management and Coordinating Center

Principal Investigator	Institute	Organ Focus
Ziding Feng, Ph.D.	Fred Hutchinson Cancer Research Center, Seattle, WA	All sites
Dan Crichton, M.S.	National Aeronautics and Space Administration, California Institute of Technology, Pasadena, CA	All sites

Network Consulting Committee

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Houston, TX

Sustaining the Scientific Direction

In recent decades, scientific research has deepened our insights into numerous biological processes. The draft map of the human genome was presented just two years ago. While such research proceeds at a rapid pace, the translation of these discoveries into medical benefits has yet to catch up. The Early Detection Research Network seeks to harness the emerging scientific knowledge into practical clinical tests.

Discovery, Evaluation, and Validation through Collaboration

The Network promotes collaboration among researchers by creating an investigator-driven environment of "cross-fertilization," that is, teamwork across disciplines and laboratories to achieve common goals. These objectives are to:

- Develop and test promising biomarkers or technologies in institutions with the scientific and clinical expertise to obtain preliminary information that will guide further testing;
- Efficiently evaluate promising, analytically proven biomarkers or technologies, including measures of diagnostic predictive accuracy, sensitivity, specificity, and, whenever possible, medical benefits as predictors of clinical outcome or surrogate endpoints for early detection and for prevention intervention clinical trials;
- Analyze biomarkers and their expression patterns, including simultaneous analyses of multiple markers which will serve as background information for subsequent large definitive validation studies in the field of cancer detection and screening;
- Collaborate among academic and industrial leaders in molecular biology, molecular genetics, clinical oncology, computer science, public health, and other disciplines, for the development of high-throughput, sensitive assay methods for biomarkers for early detection and risk assessment;
- Conduct early phases of clinical and epidemiological studies; and
- Encourage collaboration and rapid dissemination of information among grantees to ensure progress and avoid fragmentation of effort.

As the integration of disparate groups and work elements proceeds in a collaborative project, a major role for the National Cancer Institute is to motivate all stakeholders toward a common goal. The Network's vertical approach encourages researchers to work in teams as collaborators rather than as individual competitors. Efforts focus on ensuring a smooth flow between laboratory discoveries and clinical translation of biomarkers to provide timely, cost-effective tests for early detection of cancer and identification of high-risk individuals.

Successful teamwork is predicated by well-defined workflow, collaboration, and communication. People with similar areas of research (such as in the Organ Site Collaborative Groups), and people who have roughly similar time horizons for their output need to work together. Laboratories that are developing biomarkers gain much by sharing information on both successes and failures.

Guiding Principles for Biomarker Research

The Network has developed and implemented systematic, comprehensive guidelines to develop, evaluate, and validate biomarkers. This five-phase approach establishes both a standard and a road map for successfully translating research on biomarker applications from the laboratory to the bedside.

Phase 1 includes exploratory study to identify potentially useful biomarkers–this is called the "discovery" phase.

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

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.

Phase 5 is when large-scale population studies evaluate not just the role of the biomarker for detection of cancer, but the overall impact of screening on the population.

Although the Network's focus is projected mainly on phases 1 through 3, researchers have welcomed the five-phase structure because it provides an orderly series of studies that build upon each other to yield an efficient and thorough approach to biomarker development. The key aspects of study designs for each of the phases has been discussed and published. (See Appendix II.)

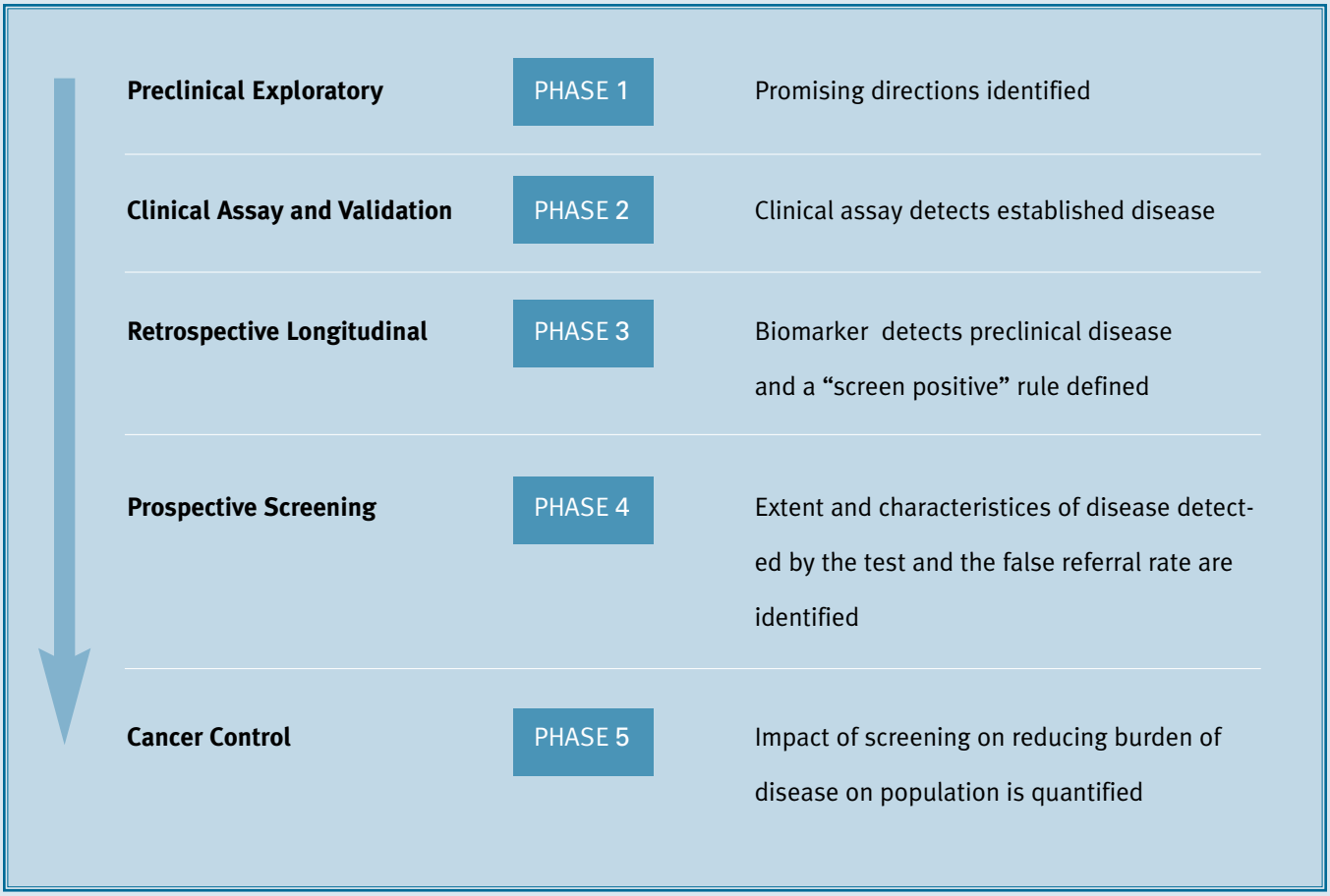
In contrast to the development structure, literature related to the statistical evaluation of biomarkers is both limited and scattered. The Data Management and Coordinating Center and Fred Hutchinson Cancer Center are advancing several concepts and methods to fill a major gap in research methodology for studies in phases 2 to 5. This is already proving to be a valuable resource.

Specifically, these investigators have used "Boosting Tree" algorithms, developed in-house, to analyze data from tests of tissues from patients with prostate cancer, benign prostatic hyperplasia, and healthy cells at the Eastern Virginia Medical School. The tests, known as Surface-Enhanced Laser Desorption/Ionization or SELDI, enable the detection of a specific protein pattern for each of the three groups with sensitivity and specificity of 97%.

Based on these results, a validation study using samples from NCI's Prostate Cancer Prevention Trial was proposed. In this clinical trial, 18,000 men took either the drug finasteride or a placebo for seven years and then received a prostate biopsy. In the Network proposal, testing these biopsy samples would be the phase 3 study that moves forward based on data provided by a phase 2 study–the study using samples from Eastern Virginia Medical School. The phase 3 sample size was calculated based on joint confidence regions of sensitivity and specificity, a novel approach developed by Data Management and Coordinating Center investigators.

Phases of Early Detection

The Network focus on the first three phases of early detection has established a well documented scientific foundation for research to follow in Phases 4 and 5.



Source: Journal of the National Cancer Institute, *Vol. 93, No. 14, 1054-1061, July 18, 2001*

Measuring the Predictive Power of a Biomarker

NCI investigators have developed guidelines for statistical design and analysis of nested case-control studies on serially collected blood or tissue specimens. These guidelines, listed below, will be used by Network 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 combination of individual markers and changes in marker levels over time.
- To avoid over-fitting, the criteria for a positive marker combination developed in a training sample should be evaluated in a random test sample from the same study and, if possible, a validation sample from another study.
- To identify biomarkers with true and false positive rates similar to mammography, the training test and validation samples should each include at least 110 randomly selected subjects without cancer and 70 subjects with cancer.

Source: BMC Medical Research Methodology 2:4, 2002, online BioMed Central, http://www.biomedcentral.com/1471-2288/2/4

Progress in Discovery by Organ Site

The Early Detection Research Network is actively testing and evaluating numerous biomarkers to detect cancer or cancer risk in a variety of organ sites. The initial phase of the process encompasses discovery–identifying potential biomarkers and making initial inroads in evaluating and determining whether or not they might be a useful test.

The following pages detail some of the most promising results of this discovery phase. The sections are organized by the organ-specific collaborative groups used in the Early Detection Research Network to facilitate collaboration and information exchange.

Breast and Gynecologic Cancers

BREAST CANCER

Breast cancer is the most frequently diagnosed cancer and the second most frequent cause of cancer death for women in the United States. More than 203,000 women are diagnosed with breast cancer each year. Mammography is an important and useful early detection test for breast cancer, but it is not the final answer. While nearly two-thirds of breast cancers are diagnosed while localized, more than one-third are not. Women diagnosed with breast cancer at the most advanced stages have less than a 25% chance of living for five years. Biomarkers to detect breast cancer and breast cancer risk will play a crucial role in decreasing the number of women who are diagnosed with or die from this disease.

- Investigators at Duke/Abbott Developmental Laboratory analyzed the expression of two promising markers for the detection of breast cancer, *BS106* and *BU101* in tissues from early stage and metastatic breast cancers, along with the expression of two known markers, *cytokeratin 19* and *mammaglobin*. The data showed that cytokeratin-19 is as good as or better than all other markers in detecting a breast cancer when it is present, but is not good at detecting risk when a woman does not have the disease. Of the breast-specific markers, *BS106* appears to be the best marker to move forward into more research.

- Collaborative studies between Duke University and Eastern Virginia Medical School are testing whether SELDI can detect circulating levels of the protein markers, *BU101* and *BS106*. These investigators have successfully used the SELDI approach for analyzing antibody-mediated prostate-specific antigen *(PSA)* detection. A second study will explore the possibility of early detection of breast cancer using artificial intelligence to detect SELDI profiles. A series of blood serum specimens from benign and malignant breast patients are being analyzed to see if this technique can successfully distinguish between cancer and benign disease.

What is SELDI?

Surface-Enhanced Laser Desorption/Ionization or SELDI is a process that enables the exploration, mapping, and discovery of molecular actions. It is a chip-based molecular imaging process where silicon wafers used in computers are coated with an active layer of molecular "bait" designed to capture DNA or proteins. These chips are exposed to the materials under study. The image of molecules captured on the chip's surface is developed and can be "read" with laser energy to create a digital image or molecular map of proteins. Within the Network, investigators are looking to see how the molecular map of proteins changes between healthy tissues, precancerous tissues, and cancer.

Source: LumiCyte, Inc., http://www.lumicyte.com/html/technology/2/.

- Eastern Virginia Medical School investigators in collaboration with Duke/Abbott Developmental Laboratory and other Network members are also evaluating the SELDI protein-profiling assay as a serum-based diagnostic tool for breast cancer. A pilot study involving the analysis of serum samples from 40 newly diagnosed patients with breast cancer and 40 patients with benign breast diseases showed breast cancer was detected with 92% sensitivity and 70% specificity. A larger-scale study including the analysis of 300 serum samples from the collaborating universities is in progress.

Data Summary of Promising Breast Cancer Biomarkers

Marker	*Cytokeratin-19*	*Mammaglobin*	*BU101*	*BS106*
Sensitivity (cancer)	95/95 (100%)	90/95 (95%)	82/95 (86%)	94/95 (99%)
Units (SKBR3 eq)	0.001-100	0.0008-130	0.017-1800	0.06-5900
Range	1 x 105	1.6 x 105	1.06 x 105	9.8 x 104

- Investigators at the Johns Hopkins University, Food and Drug Administration, National Cancer Institute, and Eastern Virginia Medical School are jointly developing a "proteomic profile" using SELDI for early detection of breast cancer. By comparing samples of breast fluid (or blood) from women with early, curable cancers to samples from women with normal breasts, they were able to identify specific protein patterns that consistently appeared in one group but not the other.

OVARIAN CANCER

Ovarian cancer is the leading cause of death from gynecological malignancies and is the fifth most common female cancer in the United States. Network and NCI investigators are developing a novel approach in their quest for identifying ovarian cancer at the earliest stage. Unfortunately, only 25% of patients are diagnosed when ovarian cancer is still localized to the ovary. Up to 90% of these very early cancers can be successfully treated, while only 30% of the patients with more advanced cancers will survive five years.

- Investigators from the Brigham and Women's Hospital in Boston have successfully used cDNA microarray analysis to compare ovarian cancer epithelial cell lines and normal surface epithelial cells to identify genes that are upregulated in cancer. Two genes, *prostasin* and *osteopointin*, are the newest candidate markers for ovarian cancer and may prove complementary to *CA125*, an ovarian cancer biomarker that is used primarily in managing treatment of the disease, but is also being evaluated as a cancer screening test.

  Prostasin is normally secreted by the prostate gland, but has been found to be elevated in epithelial ovarian cancers; tissues from 64 cases of ovarian cancer showed nearly twice the level of prostasin compared to normal tissues.

  Osteopontin is found in body fluids and extracellular matrix components. In 51 ovarian cancer cases, the levels of osteopontin were three times the average level in 107 healthy control subjects.

What is microarray analysis?

Significant information about potential biomarkers is being gathered by microarray analysis of gene and protein expression. Microarray technology is a powerful, but technically challenging, new research tool that allows scientists to assess the level of expression of a large subset of the 60,000 human genes in a cell or tissue. This technology can quickly produce a snapshot of the genes that are active or inactive in tumor cells, preneoplastic cells, or healthy cells, providing critical information in narrowing down the precise molecular triggers of the cancer process.

- Investigators at Northwestern University, Chicago, have found that lysophosphatidic acid *(LPA)* is elevated in the plasma of women with ovarian cancer, including 90% of women with stage I disease. The presence of *LPA* in early stage disease may suggest that it is produced by the cancer itself and plays a role in allowing the cancer to spread.

  In a collaborative study with Ataigin Technologies Inc., plasma levels of *LPA* were found to be significantly higher in ovarian cancer patients when compared to healthy female controls. In comparison, *CA125* was elevated in only 43% of women with early stage ovarian cancer using the same patient population. A trial evaluating 1,600 women will determine the sensitivity and specificity of a high-throughput version of this assay. Analysis of the data is under way.

LPA may also be involved in matrix-metalloproteinase *(MMP)*-mediated invasion and tumor spread. A recent study demonstrated that *LPA* added to ovarian cancer cells up-regulated activation of certain *MMP*s involved in the migration of cancer cells and their invasion into healthy tissues.

All three of these prospective markers, *prostatin*, *osteopontin*, and *LPA*, will be assessed using a joint set of specimens from women with ovarian cancer and healthy controls to compare their potential.

- Several EDRN laboratories and centers in collaboration with the Food and Drug Administration and NCI are conducting studies to validate the accuracy, and reproducibility of SELDI technology with the Standard Reference Materials (SRM) 1951A for serum protein provided by the National Institute of Standards and Technology. Upon completion of this analytical validation, investigators are proposing to assess proteomic patterns as a screening tool for earlier stages of ovarian cancer in women at increased risk of developing ovarian cancer.

- A Network study led by investigators at the National Institutes of Health described the application of an artificial intelligence algorithm to analyze proteomic data to identify diagnostic patterns that could distinguish blood from women with and without ovarian cancer. Rapid, high-throughput molecular weight profiles consisting of tens of thousands of data points were generated by SELDI and analyzed by a novel, heuristic pattern recognition algorithm. The trained algorithm was accurate in predicting the presence of ovarian cancer in 260 of 260 women, including 60 of 60 from sera of women with stage 1 cancer. Validation of this new tool is ongoing in larger study sets of serums for diagnostic utility for early detection of prostate, ovarian, breast, and colon cancer.

- Ovarian cancer may be due to a mutation in either the *BRCA1* or *BRCA2* genes, which are more commonly associated with risk of breast cancer. Two mutations in *BRCA1* (185delAG and 5382insC) and one in *BRCA2* (6174delT) are common in the Ashkenazi Jewish population and each mutation is associated with an increased risk of ovarian cancer. A significant proportion of Jewish women with ovarian cancer may carry one of these mutations.

  Recent publication by Northwestern University investigators reported that approximately 40% of ovarian cancers in Ashkenazi Jewish women with no family history of breast or ovarian cancer showed the presence of a founder *BRCA1* or *BRCA2* mutation (an inherited germline mutation). Also, the investigators revealed that about 20% of breast cancer patients in this group had a founder mutation in *BRCA1* and or *BRCA2* genes. Screening for a founder mutation in *BRCA1* and *BRCA2* might greatly benefit early detection of ovarian and breast cancer in high-risk women.

- Investigators at the Brigham and Women's Hospital reported that the application of SELDI to profile proteins, which are detected in blood sera of ovarian cancer patients as compared to controls, led to the identification of a marker. The protein was purified and identified as the alpha chain of hepatoglobin and was found to be significantly elevated in 94 cases of ovarian compared to 99 healthy controls. As an acute-phase reactant protein, alpha hepatoglobin may be a general marker for cancer and therefore have applicability in the detection of disease in several organs.

- Investigators at the Mayo Clinic have identified a novel isoform of *EGFR*, referred to as *sErbB1* p110. Pre-operative levels of this protein were significantly lower in women with stage III or IV epithelial ovarian cancer than in healthy women of similar ages. To determine if serum *sErbB1* may be a useful (negative) biomarker for ovarian cancer, *sErbB1* p110 levels have been compared in healthy women, women with benign masses in organs near the ovaries, other benign gynecologic conditions, and women with stage I-IV ovarian cancers. Women with ovarian cancer have a lower median serum *sErbB1* p110 level than the control groups of healthy women and those with benign masses in organs near their ovaries or other benign gynecologic conditions. A subset of ovarian cancer patients had a higher median *CA125* compared to women with benign diseases, suggesting that serum *sErbB1* p110 levels, in combination with *CA125* levels, may be useful for differentiating between benign and malignant pelvic masses.

Gastrointestinal and Other Cancers

Investigators of the Gastrointestinal and Other Cancers Collaborative Group focus on the discovery and validation of surrogate biomarkers of cancers of the colon, esophagus, liver, pancreas, stomach, and gall bladder.

COLON CANCER

Colon cancer is the third most frequently diagnosed cancer in men and women in the United States and the third most frequent cause of cancer death. 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 the 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.

- Scientists at the Great Lakes-New England Clinical Epidemiology Center Consortium identified a ligand, or binding protein, for *galectin-3* in circulating blood; *galectin-3* is a protein related to tumor progression and spread. The new ligand was found to be a hepatoglobin-related protein, and is present in higher concentrations in patients with colon cancer than in those with adenomas (precancerous polyps) or in normal subjects. There was also an increase in serum *galectin-3* itself in patients with colon cancer as compared to those with adenomas or to normal, but no correlation between the ligand and *galectin-3*. In the blood of people with colon cancer, there is twice as much of any haptoglobin than in the blood of normal people, and 30 times more of the ligand. This difference in concentration suggests that cancer causes alterations in how haptoglobins work in the body and may mean that haptoglobins are good biomarkers for early cancer detection.

- Network scientists at Creighton University, in collaboration with Exact Laboratories, are examining whether a stool-based assay for mutated *BAT-26*, a marker of microsatellite instability, could be used to diagnose early stage colorectal cancer in people with symptomatic Hereditary Non-Polyposis Colorectal Cancer (HNPCC). People with HNPCC do not have an unusual number of polyps (which would signal cancer risk) and usually develop a solitary colorectal tumor, often in the right (proximal) side of the colon (most

cancers occur on the left or distal side). Since many proximal cancers exhibit microsatellite instability, it was hypothesized that detection of a specific one might complement colorectal cancer screening, especially if the marker reflected specifically proximal cancers. Of 78 cases, 70 had negative *BAT-26* stool tests and, in cases where abnormal tissue was available to test, negative *BAT-26* tissue tests. In the eight positive *BAT-26* tests, those patients had a significant pathology, i.e. had a cancer or precancerous tissues in the proximal colon. Current data suggest that stool *BAT-26* testing may detect early stage colorectal neoplasia in high-risk individuals with HNPCC.

PANCREATIC CANCER

Currently there is no successful treatment for pancreatic cancer, making it one of the most lethal of all human cancers. Pancreatic cancer can be successfully treated when diagnosed at its earliest stage, however only about 8% of cases are diagnosed this early. Families have been identified where pancreatic cancer occurs frequently and researchers have identified several genes linked to the inherited risk for the disease.

- Investigators at Creighton University established a cohort containing 159 pancreatic cancer families. Hereditary pancreatic cancer is a very diverse disease which is frequently associated with inherited cancer syndromes, including familial atypical multiple mole melanoma syndrome, also known as FAMMM. In collaboration with Johns Hopkins University and the University of Toronto, Creighton investigators have discovered the *CDKN2A* (*p16*) germline mutation in eight of 20 FAMMM families in their cohort.Testing for *CDKN2A* mutations is still ongoing, but given the known genotypic and phenotypic diversity in the FAMMM syndrome, the investigators anticipate that germline mutations in additional genes will one day be identified as causing pancreatic cancer in patients without *CDKN2A* (*p16*) mutations.

Up to 10% of malignant melanoma-affected families showed patterns of transmission consistent with a hereditary (autosomal dominant) predisposition. An unknown portion of these carry the *CDKN2A* mutation of the FAMMM-PC syndrome. People who carry the *CDKN2A* mutation show a low risk of developing pancreatic cancer (about 17%) compared to as much as an 80% likelihood for developing melanoma. Thus, this FAMMM-PC model will allow cancer prevention activities for melanoma through identification of the FAMMM phenotype, defined by the presence of many moles or atypical moles. The *CDKN2A* germline mutation predisposing to pancreatic cancer will provide an opportunity for development of a variety of putative biomarkers that correlate with the *CDKN2A* mutation.

This research is expanding to other hereditary syndromes where pancreatic cancer can appear, including: hereditary non-polyposis colon cancer of the Lynch syndrome II variant (*MLH1* or *MSH2* mutation), Familial Adenomatous Polyposis with colorectal cancer and pancreatic cancer (*APC* mutation), and hereditary breast-ovarian cancer syndrome where germline markers for *BRCA1* and *BRCA2* appear to predispose to pancreatic cancer.

ESOPHAGEAL CANCER

Esophageal cancer rates are rising, mostly due to increases in adenocarcinoma of the esophagus. More than 13,000 people are diagnosed with esophageal cancer each year and 12,600 die of the disease. Five-year survival is about 13%. The only known precancerous condition that signals risk for esophageal cancer is Barrett's Esophagus or Barrett's dysplasia.

A collaborative effort between scientists at University of Michigan Biomarker Developmental Laboratory and the Great Lakes-New England Clinical Epidemiology Center focused on gene amplification in esophageal adenocarcinomas and in Barrett's metaplasia with high-grade dysplasia. A cohort of 87 esophageal adenocarcinomas and 22 cases of Barrett's metaplasia with high-grade dysplasia and 3 Barrett's adenocarcinoma cell lines were examined. One or more gene amplification events were present in 50/87 (57%) of adenocarcinomas. The researchers will look further at these events for potential biomarkers. See the Web at: https://glnecec.dartmouth.edu/.

LIVER CANCER

Liver cancer rarely causes any symptoms until it has spread beyond the liver itself. The strongest risk factor for the disease is chronic infection with hepatitis viruses. About 16,600 people are diagnosed with liver cancer in the United States each year and 14,100 die of the disease.

- A collaboration between Thomas Jefferson University and Great Lakes-New England Clinical Epidemiology Center investigators focused on early detection of liver cancer and hepatitis B in a patient population with a background of hepatitis B infection. Evidence of an inverse relationship between the abundance of serum C3 fragment and an isoform of *apolipoprotein A1* (*ApoA1*) and the risk of liver cancer has been identified in a small set of samples examined thus far. The average group intensity of C3 fragment in patients with no liver disease or with a symptomatic hepatitis B infection is greater than in the groups of those with active hepatitis of liver cancer. Overall, however, consistently low levels of both of these proteins are seen in patients with a diagnosis of liver cancer.

Lung and Upper Aerodigestive Cancers

LUNG CANCER

Lung cancer is the greatest cause of cancer death in the United States. More than 150,000 people die of lung cancer each year. There is no established early detection test for the disease, and only 15% of lung cancer cases are diagnosed when the disease is localized.

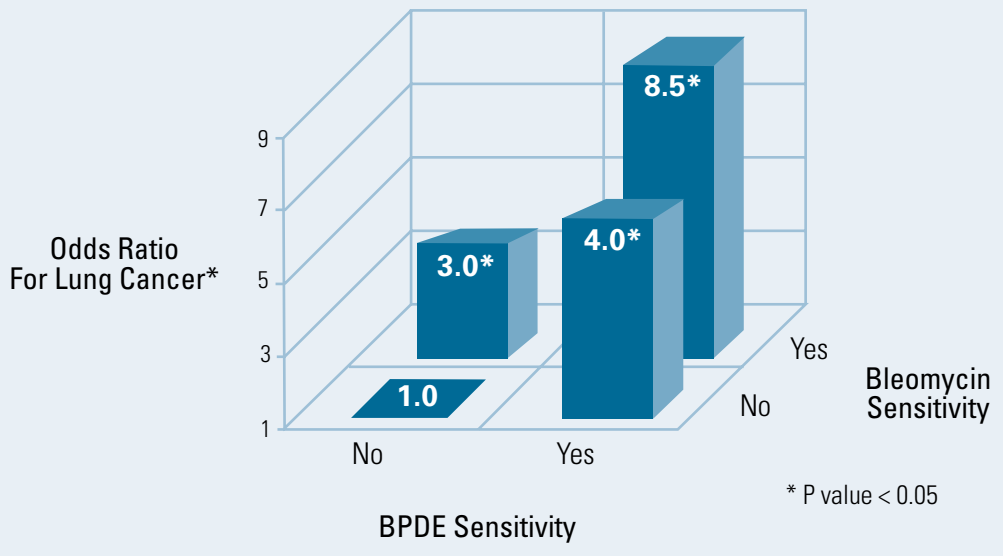
- Abnormal DNA methylation patterns are a hallmark of most cancers. Gene-specific methylation changes in tumor cells and sputum are being evaluated as promising markers of early lung cancer. Different laboratories have optimized different assays of gene-specific promoter hypermethylation. Investigators from Johns Hopkins University, the University of Texas M. D. Anderson Cancer Center, The University of Texas Southwestern Medical Center, Moffitt Cancer Center and Lovelace Respiratory Research Institute (Albuquerque), are each using their own methylation assay to determine accuracy for cancer endpoints on blinded lung cancer and control specimens. This collaborative study will determine the methylation assay that most accurately reflects the presence of lung cancer and thereby the assay to be proposed for subsequent validation.

- Investigators at the University of Michigan have compiled a database of protein expression in lung cancer that integrates two-dimensional gel profiles, mass spectrometry data, quantitative protein data, and gene expression data to serve as a resource for identification of protein markers in lung cancer. Investigators at the Moffitt Cancer Center have found specific protein profiles for microdissected lung tumors and pre-malignant lesions using SELDI.

- Investigators at the M. D. Anderson Cancer Center are examining DNA repair gene polymorphisms that may predict DNA repair capacity. Polymorphisms in the nucleotide excision repair gene, *XPD*, could have an effect on host capacity for removing bulky adducts induced by exposure to carcinogens. If true, detection of the differences in DNA repair capacity in individuals of different *XPD* genotypes would be possible. The investigators report that the variant Gln751Gln and Asp312Asn genotypes were associated with less optimal DNA repair capacity. Both cases and controls with the wild type genotypes exhibited the most proficient DNA repair capacity. The risk for suboptimal DNA repair ability (defined as less than the median value among the controls) was 1.57 for those with the Gln/Gln751 genotype. For cases with the Asn/Asn312 genotype, the risk was 3.50. For cases who were homozygous at either locus, the risk was 2.29.

The ability to identify smokers with the highest risks of developing tobacco-related cancers has substantial implications for early detection.The challenge in such risk assessment is to account for inter-individual variation in susceptibility to tobacco carcinogenesis. One such measure is the *in vitro* mutagens (e.g., BPDE and bleomycin) challenge assay that reflects the combined effects of the extent of mutagen induced initial chromosomal damage and the effectiveness of subsequent DNA repair. In a series of published studies, this assay has been shown to be a strong, statistically significant, and independent risk factor for a variety of cancers.

Risk Estimates for Bleomycin and BPDE Sensitivity



* adjusted for age, gender, ethnicity and smoking status

- Investigators from the Johns Hopkins University compared expressed genes of non-small cell lung cancer with those of normal lung using serial analysis of gene expression (SAGE) to describe a molecular signature for lung cancer. Further work is under way on this signature.

- Investigators at the University of Texas Southwestern Medical Center report a new tumor suppressor gene at 3p21.3, *SEMA3B*. The expression of this gene is frequently inactivated in lung cancers.

- University of Colorado is studying gene regulation pathways involving *HOX*, *WNT*, and fibroblast growth factor (*FGFR*), all of which are frequently altered in lung cancer, as potential biomarkers.

- Investigators at the University of Pittsburgh report that estrogen signaling plays a biological role in both the epithelium and the mesenchyme in the lung. Estrogens could potentially promote lung cancer, either through direct actions on precancerous or cancerous cells or through indirect actions on lung fibroblasts.

- Investigators at New York University have found that *p38*, a mitogen-activated protein kinase usually associated with stress responses, growth arrest, and apoptosis, is activated in all of their human lung cancer samples. This activation suggests an additional role for this pathway in malignant cell growth or transformation.

Prostate and Other Genitourinary Cancers

PROSTATE CANCER

Prostate cancer is the most frequently diagnosed cancer in American men, with nearly 190,000 men diagnosed each year and 30,200 dying of the disease. While prostate cancer is frequently present in men over age 50, many of these cases will never be lethal or even clinically detectable. The challenge for early detection is to discern latent from lethal disease.

- Researchers at the University of Texas at San Antonio are evaluating insulin-like growth factor *(IGF)-1, IGF*-binding proteins 3 and 4, and genetic polymorphisms of the androgen receptor for their potential to predict risk for prostate cancer in an ethnically varied population. These patients are also screened for prostate cancer by the *PSA* test and digital rectal exam.

- Scientists at the Johns Hopkins University are evaluating the clinical utility of *PSA*, human *kallikrein-2, B23* (a nuclear matrix protein), *p27* (a cell cycle regulator), and *Ki67* (a cell proliferation marker) as biomarkers for prostate cancer.

- Researchers at the University of Washington and Eastern Virginia Medical School are developing highly sensitive and specific assays in seminal plasma/prostatic fluid, serum, and urine that signal an early transformation event in prostate cancer.

- The University of Texas Southwestern Medical Center is utilizing analytical spectrometric and chromatographic technology to detect differences in protein expression between prostate cancer patients and normal controls, which can be used to develop biomarkers for the disease.

- A Network associate at Massachusetts General Hospital is developing and refining a serum-based, high-throughput assay that can detect glycovariants of *PSA* through a capillary electrophoresis approach. Bioinformatic tools are being applied to enhance the interpretation of existing and new assays. It is anticipated that this study will lead to the development of new methods for analyzing and managing clinical information associated with multiple biomarkers.

- Researchers at the University of Alabama at Birmingham are characterizing molecular markers in high grade prostate intraepithelial neoplasia (PIN) lesions using tissue matrix technology. Scientists at this site have the capability to validate protein biomarkers, like those identified in seminal plasma and sera.

- Investigators at the University of Texas Health Science Center in San Antonio using the San Antonio Center of Biomarkers of Risk (SABOR) cohort found that androgen receptor (*AR*)-length polymorphism is associated with prostate cancer risk in Hispanics. The shorter CAG repeat (< 18) in exon 1 of the *AR* gene confers a significantly increased risk of prostate cancer among Hispanic men compared to those with longer CAG repeats (> 18).

- The Biomarker Development Laboratory at Eastern Virginia Medical School is applying Ciphergen Biosystems ProteinChip® SELDI-TOF-MS system for discovery of the signature proteins that distinguish prostate cancer from benign prostate disorders, and healthy prostate tissue. SELDI analyses of cell lysates prepared from microdissected cells have identified clusters of small polypeptides that are overexpressed in the preneoplastic lesion PIN (prostate intraepithelial neoplasia) and continue to be overexpressed in primary and metastatic cancer cells. These small and low abundance proteins may represent significant and potential biomarkers associated with the early development of cancer.

- Coupling SELDI protein profiling of seminal plasma and serum with learning algorithms developed at the EDRN Data Management Coordinating Center at Fred Hutchinson Cancer Research Center, have achieved better assay sensitivities and specificities (i.e., >95%) than *PSA* in identifying prostate cancer.

- Using the same biochip platform, SELDI multiplex immunoassays have been constructed to quantify the prostate cancer biomarkers *PSA* and *PSMA* (prostate specific membrane antigen) in sera. A significant observation was finding serum *PSMA* superior to *PSA* in distinguishing between benign prostate hyperplasia and prostate cancer. These early results suggest the SELDI system is a promising proteomic approach for biomarker discovery and as a clinical platform for development of innovative diagnostic assays.

- Distinct molecular changes occur at each progressive stage of prostate cancer that can be studied using gene microarray technology. University of Texas Health Science Center-San Antonio investigators in collaboration with Gene Logic Inc., determined gene expression profile in prostate cancer. Analysis was performed on the Human U95 Affymetrix GeneChip7 high density oligonucleotide microrarray platform for simultaneous analysis of 60,000 fragments. RNA were

extracted from fresh prostates from areas highly enriched in tumor (>90%) and adjacent areas from the same prostates that are free of tumor. The expression data from these samples was compared to other human tissue samples using existing software tools. Hierarchal Clustering Analysis resulted in patterns that corroborates with the Principal Component Analysis. Expression profiles of these genes in over 3,000 human normal, diseased, benign, and tumor were evaluated to determine whether they are selectively up-regulated or down-regulated in prostate cancer tissues. These prostate cancer-regulated genes provide novel markers for prostate cancer, new targets for treatment, and patient stratification. Interestingly, among the upregulated genes in the Principal Component Analysis was the alpha-Methylacyl Coenzyme A racemase, which was recently reported in several publications as a tissue biomarker for prostate cancer.

BLADDER CANCER

At present, mortality from bladder cancers is high and survival is low. Techniques for early diagnosis of these cancers are extremely limited and treatment fails in 95 percent of patients with advanced disease. Bladder cancer is the fourth most frequently diagnosed cancer in men and the tenth in women.

- University of Texas M. D. Anderson Cancer Center investigators developed a progression model for bladder cancer. The investigators determined a pattern of loss of heterozygosity in multiple loci, on multiple chromosomes, analyzing large number of neoplastic and pre-neoplastic lesions obtained from 5 cystectomies. Using this approach they attempted to identify chromosomal regions which were involved in early occult phases of bladder neoplasia and those that could be relevant for the development of more advanced disease such as severe dysplasia or carcinoma *in situ* progressing to clinically aggressive invasive cancer. In preliminary studies, a panel of 20 markers located in chromosomal regions involved in early phases of bladder neoplasia was used to profile the pattern of loss of heterozygosity on voided urine samples of patients with a history of bladder cancer that were clinically cancer free. Using this approach they were able to identify nearly 80% of patients clinically occult phases of bladder neoplasia. These markers might be used for detection and monitoring of bladder cancer.

Meeting the Challenges

By following the principles and theories of systems biology, in which disciplines like biology, chemistry, computational science, and clinical sciences are integrated seamlessly, the Network is meeting the scientific challenges of biomarker research. Interdisciplinary teams of investigators are tackling these critical areas:

- Creating novel approaches to validation studies;
- Improving informatics and information flow;
- Standardizing data reporting; and
- Creating general statistical and computational tools and standardizing reagents and assays.

Creating Novel Approaches to Validation Studies

A fundamental goal of the Network is to find methods other than randomized clinical trials to evaluate and validate biomarkers for clinical application during the early stages of investigation. Validation of new biomarkers by traditional clinical trials will be unfeasible due to cost, time required for follow-up, rapidly changing technologies, variations in treatment, and the large numbers of biomarkers expected to be discovered.

The Network's three validation laboratories remain integral to the search. A resource for all investigators in the consortium, the laboratories are housed at: the National Institutes of Standards and Technologies (NIST); University of California at Los Angeles (UCLA); and University of Alabama at Birmingham (UAB). The following studies are at different stages of validation.

Detecting Promoter Methylation as a Risk Marker

Epigenetic changes, such as methylation of the promoter regions of some key genes like *p16*, *APC*, *RAR-beta*, *DAPK*, and *MGMT*, commence early during the lengthy preneoplastic process, and thus may be useful markers for risk assessment. These markers are undergoing the Phase II validation process. Methylation is a way that a cell may temporarily or reversibly silence the activity of a gene. Many CpG regions within the human genome help regulate gene function.

This preliminary study will identify an optimal approach for the detection of promoter CpG methylation that can be recommended to a Biomarkers Validation Laboratory for a full scale study. The optimum approach will be selected based upon assay accuracy (sensitivity and specificity) relative to the presence or absence of cancer at the cellular level.

In lung cancers, methylation is present more frequently in non-small cell lung cancer tumors (43%) and cell lines (50%) than in small cell lung cancer cell lines (20%). To avoid inter-lab and intra-lab variation in promoter hypermethylation in non-small cell lung carcinoma, six Network investigators are involved in testing blinded lung tumor and control specimens to evaluate for promoter hypermethylation. A minimum of four genes will be tested and hypermethylation frequency of each gene will be measured in cell-type specific tumor specimens compared to normals to determine whether methylation detection differs by method, primers, fixation, or plasma as compared to tumor tissue.

"FISH"ing for Chromosomal Breakage

Non-random chromosomal rearrangements, including translocations, inversions, deletions and chromosomal breaks, are highly associated with cancer development.

A group of MD Anderson Cancer Center investigators has proposed to use the cancer treatment antibiotic bleomycin to induce chromosome breakage as a susceptibility marker for lung cancer. To further refine this assay, they have focused on two areas of the karyotype, or chromosomal complement of an individual, where specific chromosome damage is induced: chromosome band 3p14 and chromosome 5 (5p14 through 5q34).

NIST is validating the chromosomal breakage as a marker of lung cancer susceptibility and early lung cancer detection using Fluorescence In Situ Hybridization (FISH). Although FISH technology was reproducible, criteria for cell selection and classification of aberrations varied among observers. To standardize and evaluate criteria, a Web-based validation study was performed. Two hundred FISH images were collected in CD-R format and assigned random identification numbers. Detailed criteria were developed for cell selection from the test image collection and for cell classification of un-scored, normal, or abnormal subsets.

Eight observers scored NIST FISH Image Set 02-01 based on criteria from NIST and MD Anderson. All observers were certified. Half were doctoral-level experienced cytogeneticists. All participants had 5 years of experience. Questions addressed were: (1) given standard criteria, how similarly did cytogeneticists choose cells and score chromosome damage; (2) did concordance vary with educational level, years experience or professional certification?

Results indicate a greater degree of consensus on population chromosome damage rates than on selection of individual cells judged adequate for analysis. These results will be used to estimate the components of assay variance that can be attributed to definitions, technical variables or biological variables. Once the standard cell selection and scoring criteria are established, work on the analytical and clinical validation will proceed.

Mutations in Mitochondrial DNA as an Early Detection Marker

The mitochondrion structures within cells generate energy for the cell and contain genetic material and enzymes important for cell metabolism. Investigators at Johns Hopkins University have shown various DNA sequence mutations in cancer mitochondrial cells from lung cancer. The cancer samples also yielded a higher proportion of homoplasmic cells, that is, cells containing a single mitochondrial DNA (mtDNA) sequence species.

To validate mutations and homoplasmy, NIST was directed to sequence completely the normal mtDNA and paired cancer cell mtDNA from 200 patients. Reference threshold concentrations for mtDNA below which mtDNA cannot be measured and sequenced are being established for mutational analysis in the D-loop of mtDNA, which is known to be mutated in early stages of lung, head and neck, and bladder cancer.

NIST designed a set of mtDNA amplification primers and sequencing primers, and established conditions suitable for limited (10 - 20 ng) samples, including procedures for fluorescence sequencing that incorporate robotic workstations. Because of ethnic group-specific sequence differences, primers had to be adapted for use on five cell line controls representing both sexes and various ethnic groups reflecting the diversity of the U.S. population.

To date, background studies have refined the system and clinical work on 200 paired samples has begun. It is expected NIST will do primary amplifications and generate sequencing templates and that a contract with an off-site DNA sequencing company will perform automated sequencing. NIST will then accept, process, and analyze raw data using informatics.

Microsatellite Instability (MSI) as an Early Detection Marker

Investigators at Johns Hopkins University have developed an assay system to detect instability in microsatellites, repetitive stretches of short sequences of DNA. Microsatellite Instability (MSI) testing offers potential for improving the early detection of bladder cancer.

Bladder cancer is a common malignancy in the United States with more than 50,000 new cases in 2001. It particularly affects smokers and often follows a chronic, relapsing clinical course. Current methodologies for detecting primary or recurrent bladder cancer are not optimal in terms of sensitivity, specificity, and cost. Because patients at high risk for initial or recurrent bladder cancer require frequent surveillance, there is a great need for additional methods of early detection. Early detection may also increase the proportion of patients for whom preservation of the bladder is possible.

Validation of MSI tests for early detection of primary or recurrent bladder cancer will pave the way for its acceptance in the clinical setting. More than 10 institutions, including Johns Hopkins, MD Anderson Cancer Center, and NIST, are participating in this validation study.

Telomerase Activity as an Early Detection Marker

Telomerase is an enzyme found at elevated levels in malignant cells that allows cells to proliferate indefinitely. This activity occurs in a variety of tumors and in nearly 90% of all tumor types tested to date.

University of Maryland investigators presented preliminary data suggesting that serum telomerase levels might be a useful marker for early cancer detection. All previous measurements of telomerase activity have used cellular material, making the current assay cumbersome and reliant on slab gels and radioisotopes. NIST developed an assay based on the use of fluorescence marker and capillary gel electrophoresis. These changes considerably increased sensitivity of the assay.

The next step is to adapt the assay for high throughput analysis by incorporating the NIST robotics workstation in sample preparation and by adapting the current capillary electrophoresis protocol for high throughput on a 16 capillary ABI 3100 DNA sequencing instrument.

Improving Informatics and Information Flow

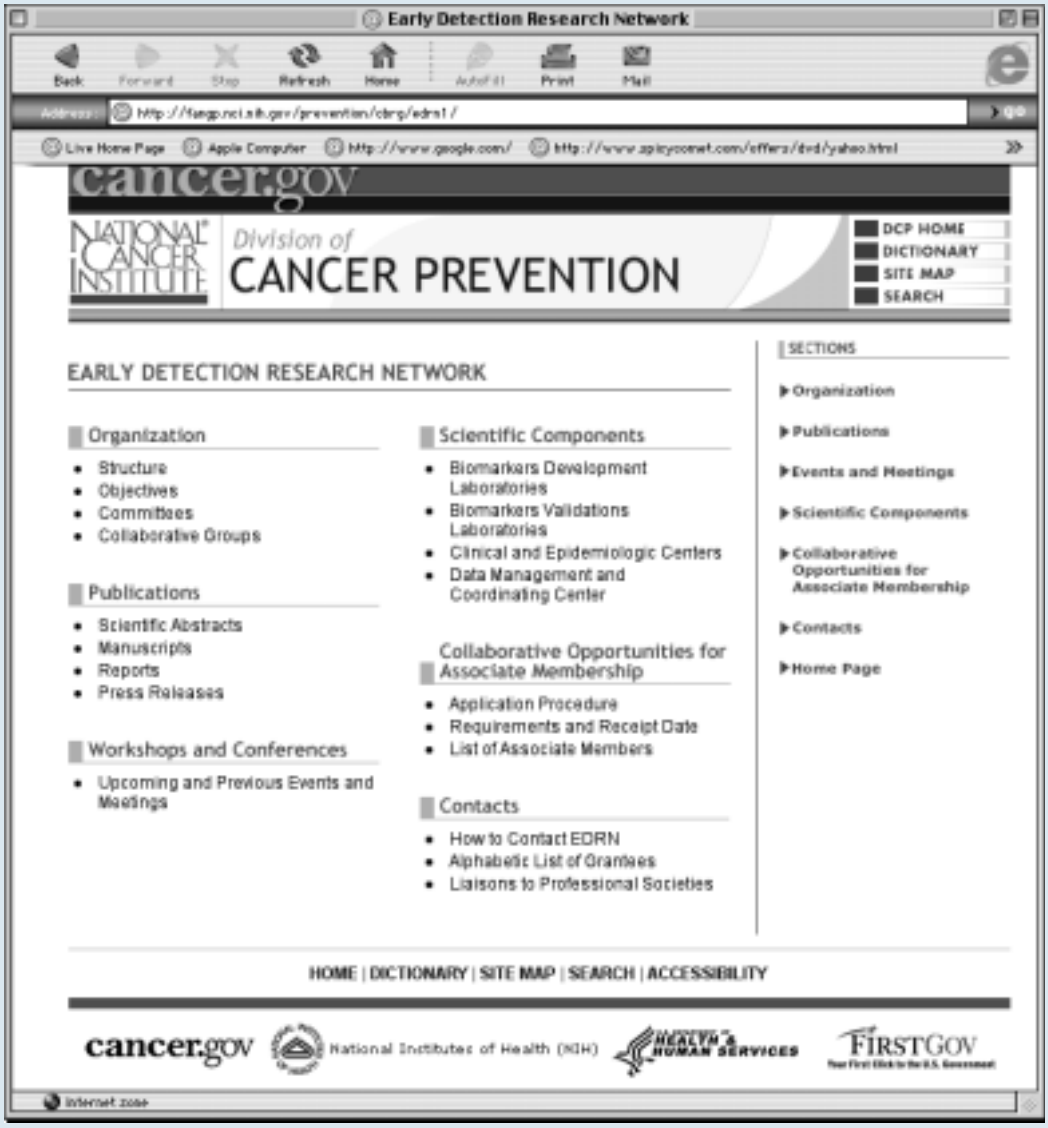
Electronic communications are making possible unprecedented research opportunities by increasing the efficiency of knowledge dissemination. With investigators distributed across the country and abroad, effective information sharing about the resources, tools and reagents available is critical to the success of the Early Detection Research Network.

By collaborating with the National Aeronautics and Space Administration's (NASA) Jet Propulsion Laboratory (JPL), the Network has launched informatics infrastructures aimed at speeding the discovery process, translating the best discoveries into clinical practice, and facilitating communications.

Network Web Sites

The public Web site (http://www.cancer.gov/prevention/cbrg/edrn/) provides information and news to scientists and non-scientists about the Network generally, recent publications, and contact information. This site, hosted by NCI, also offers non-Network investigators information about the program, such as application processes and deadlines for Associate Membership, recent Steering and Advisory Committee meetings, and scientific workshops.

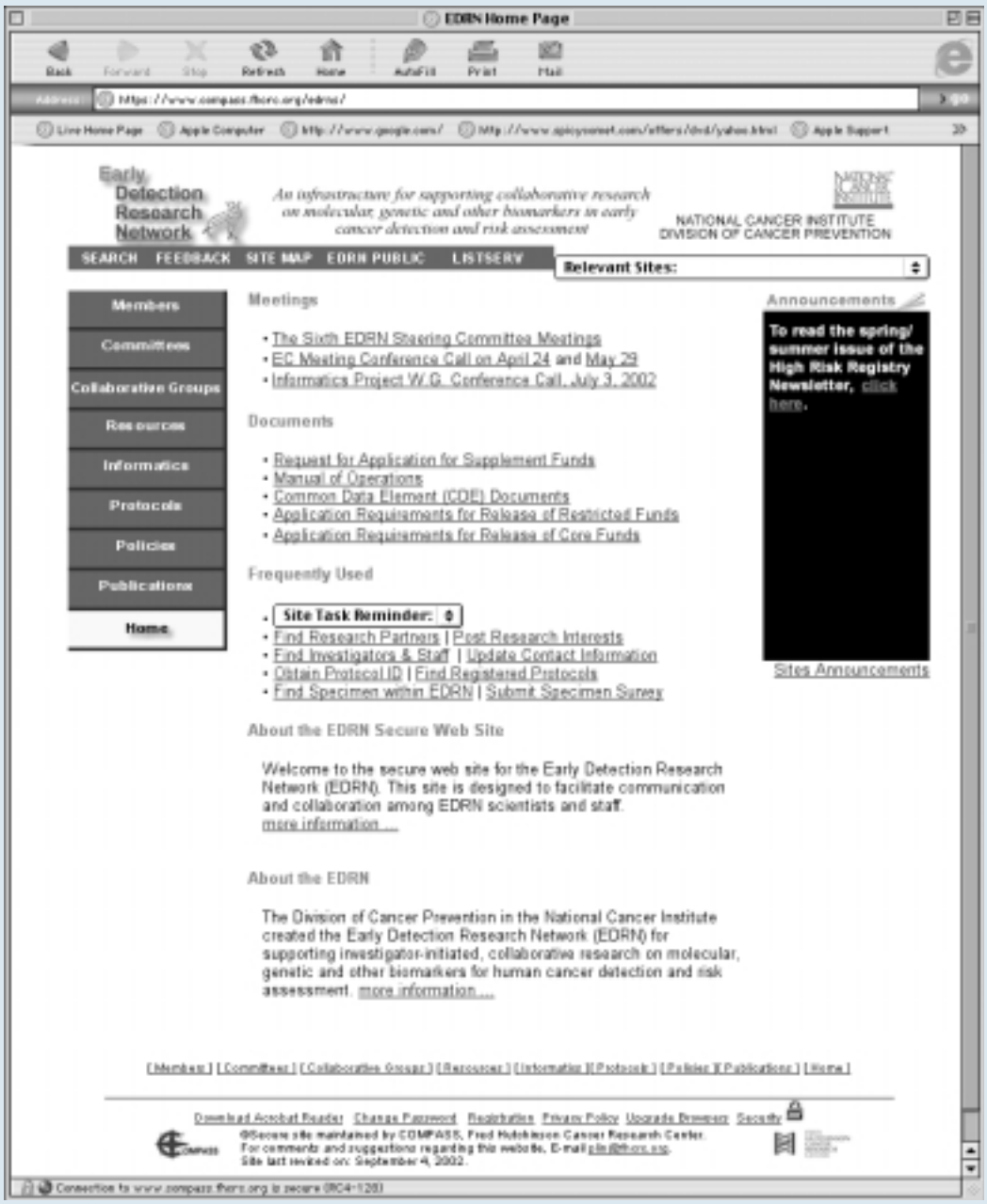
Public Web Site



The public site provides information and news to scientists and non-scientists.

The successful communication at the heart of the Network's collaborative efforts stem from the information management system implemented and maintained by the Data Management and Coordinating Center via the Network's secure Web site.

Secure Web Site



Approved applicants can use the secure site.

The secure Web site, hosted by the Data Management and Coordinating Center, is only accessible to approved applicants. Security is maintained in many ways, such as a procedure for deactivating and deleting accounts that have been unused for six or 12 months. Features of the site include:

- A database that allows investigators to obtain a protocol ID, enter their protocol abstract, and track and search Network protocols, collaborations, and IRB approval;
- Contact information for all Network sites that can be searched or updated;
- Listserv utilities to view archived emails and all subscribers of a list;
- Search capability by research interests, keywords, and Network site;
- A review system for the Standing Review Group to oversee proposals for associate Members and other reviews;
- Registration for Steering Committee meetings; and
- A reminder system for tasks that need to be competed (such as entering protocols on the Web site to obtain a protocol ID, entering research interests, or completing a survey).

Standardization of Data Reporting

The Network is one of the pioneers in developing common data elements (CDEs) to speed consistency in the way data are described across institutions. With improved data classification, scientists have the capability to use common search criteria and common data elements to retrieve information.

A complete set of CDEs is available to be collected for all Network studies; drafts of 10 different sets of specimen-specific CDEs; and five different sets of organ-specific CDEs. Eight sets of the specimen-specific CDEs and three sets of the organ-specific CDEs are being piloted by Network sites.

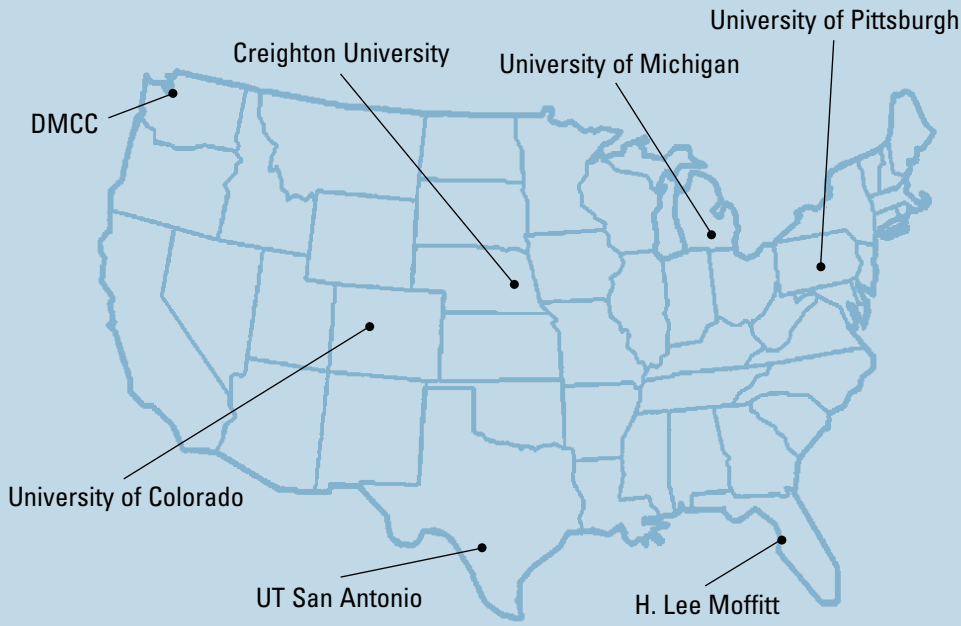
Coordinating the development of CDEs included building a data dictionary compliant with ISO/IEC 11179 database guidelines. Part of this effort focused on developing an online database on the Network's secure Web site to facilitate use of CDEs by investigators to track modifications and version changes.

Early Detection Research Network Exchange (ERNE)

NCI and NASA's Jet Propulsion Laboratory (JPL) entered into an interagency agreement to research and implement informatics solutions across the Early Detection Research Network to enable data sharing for geographically diverse science collaborations. JPL, part of the California Institute of Technology, is NASA's lead center for robotic exploration of the solar system, and has put into practice data archiving and distribution systems to support planetary science for the past 15 years.

JPL and the Data Management and Coordinating Center lead the development of the Early Detection Research Network Exchange (ERNE). This system is both a distribution and computing network that allows remote access to live databases at each Network site via the secured Web site. There is minimal impact on the local site.

Network Sites Participating in ERNE Pilot Project



Potential application of the system is broad. The NCI/JPL collaboration enables common approaches to the establishment of data architectures that will benefit both biomedical and space science research. These architectures provide tools to locate, access, and exchange information across widely distributed databases. As such, opportunities arise for new science analysis and discoveries through the increased volume and diversity of data that is captured and shared.

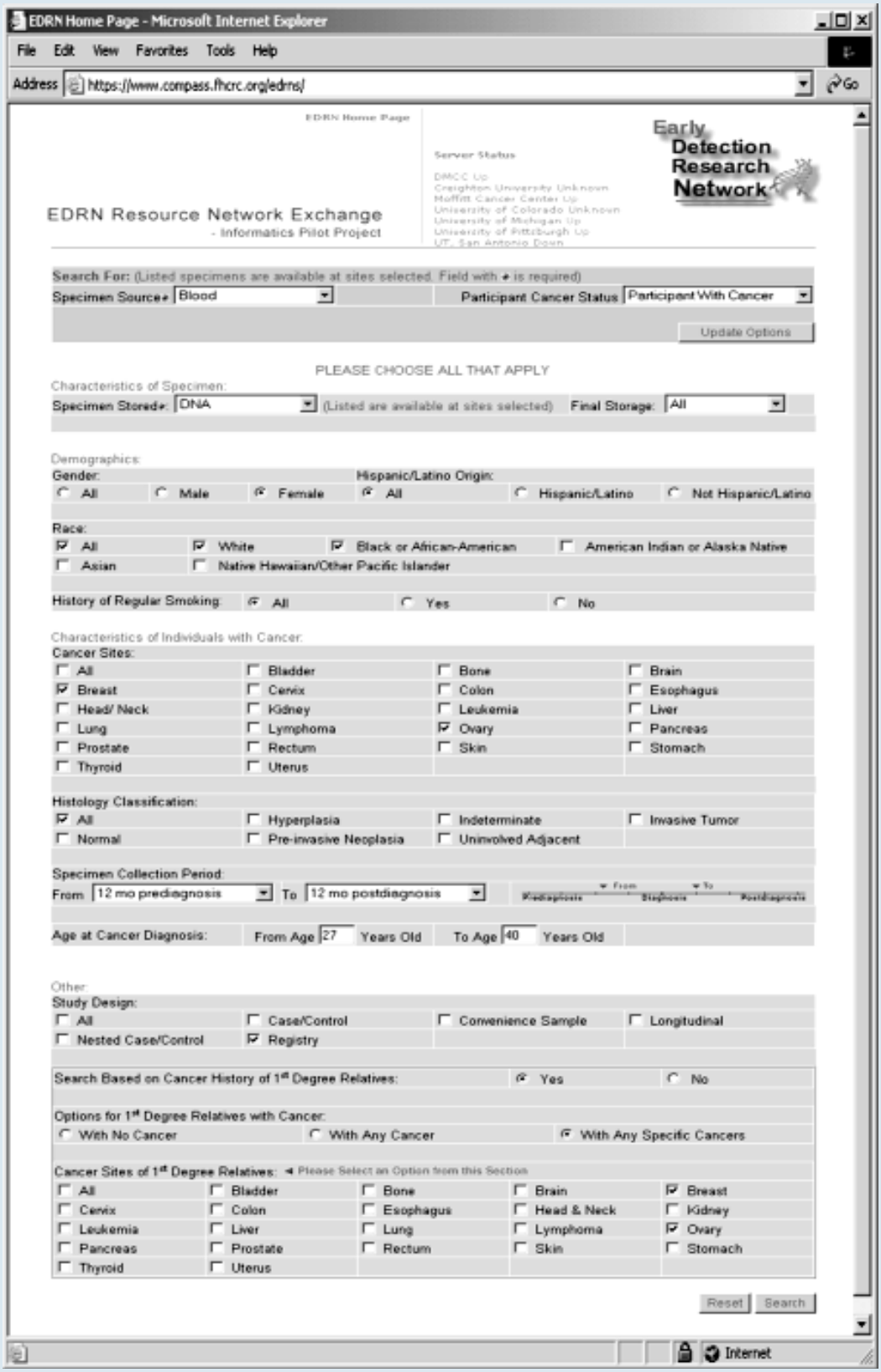
The JPL architecture links existing institutional databases containing specimen information into a single Web-enabled tracking system, which complies with data security requirements in the 1996 Health Insurance Portability and Accountability Act.

Users can search databases through the system for a variety of different types of specimens based on specific epidemiological and clinical characteristics of the specimen donor. This specimen identification system successfully connected to live databases from two Network sites during the feasibility phase via a query user-interface on the secure Web site.

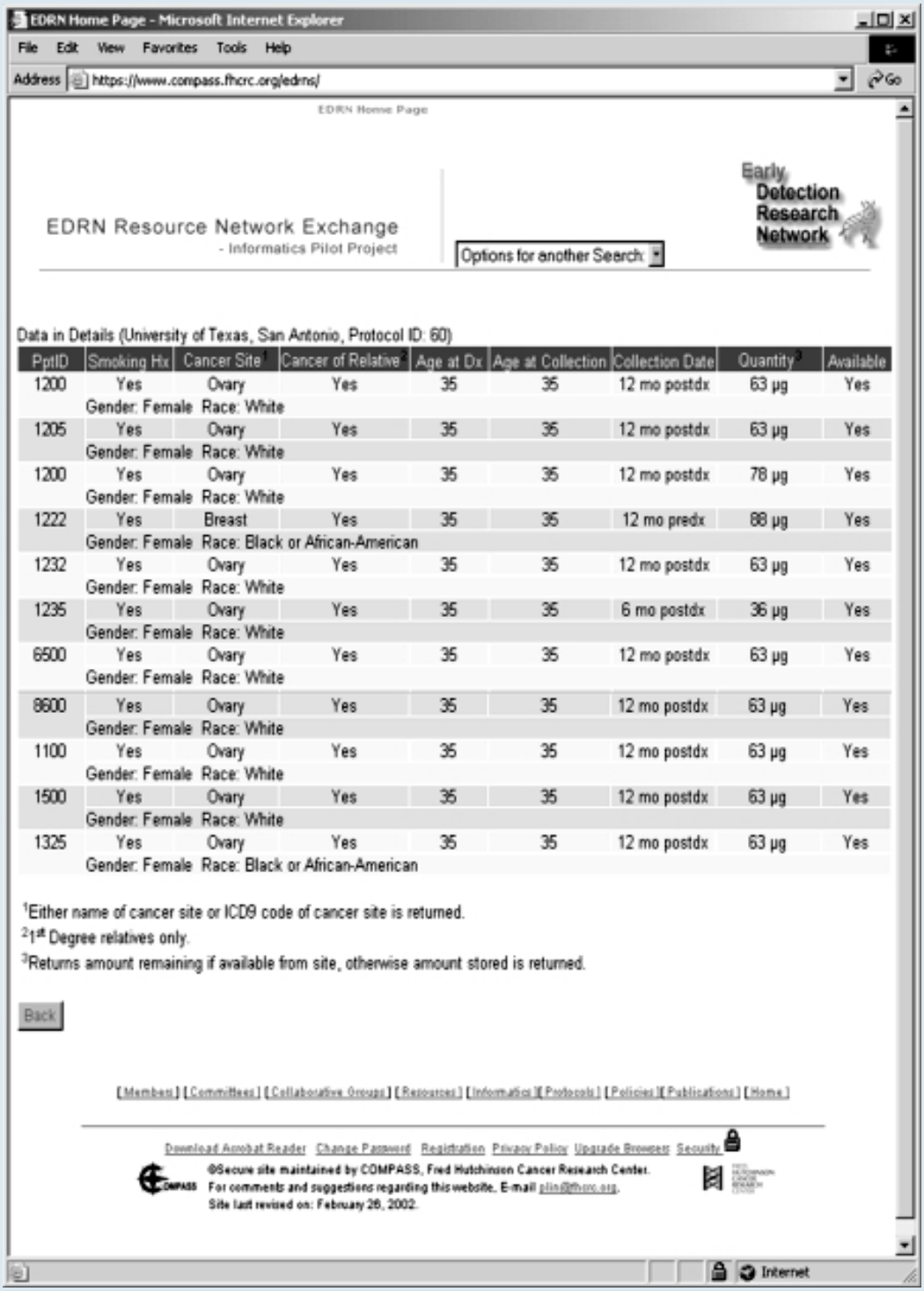
The pilot phase, scheduled to be completed in Fall 2002, involves adding databases from four more Network sites; assessing the ability of the system to expand to additional sites; improving the query user-interface; and developing tools to facilitate additional sites.

ERNE allows Network investigators to create detailed epidemiological queries of tissue specimens, bodily fluids, purified nucleic acids, and whole cell lysates from specimen collections/repositories across the Network for biomarker discovery and validation studies. The epidemiological and clinical data elements selected to create queries were based on the Network's common data elements.

ERNE Informatics Pilot Project Query Request



ERNE Informatics Pilot Project Query Result



EDRN Home Page - Microsoft Internet Explorer

File Edit View Favorites Tools Help

Address https://www.compass.fhcrc.org/edrns/

EDRN Home Page

EDRN Resource Network Exchange
- Informatics Pilot Project

Options for another Search:

Early Detection Research Network

Data in Details (University of Texas, San Antonio, Protocol ID: 60)

PptID	Smoking Hx	Cancer Site[1]	Cancer of Relative[2]	Age at Dx	Age at Collection	Collection Date	Quantity[3]	Available
1200	Yes	Ovary	Yes	35	35	12 mo postdx	63 µg	Yes
	Gender: Female	Race: White						
1205	Yes	Ovary	Yes	35	35	12 mo postdx	63 µg	Yes
	Gender: Female	Race: White						
1200	Yes	Ovary	Yes	35	35	12 mo postdx	78 µg	Yes
	Gender: Female	Race: White						
1222	Yes	Breast	Yes	35	35	12 mo predx	88 µg	Yes
	Gender: Female	Race: Black or African-American						
1232	Yes	Ovary	Yes	35	35	12 mo postdx	63 µg	Yes
	Gender: Female	Race: White						
1235	Yes	Ovary	Yes	35	35	6 mo postdx	36 µg	Yes
	Gender: Female	Race: White						
6500	Yes	Ovary	Yes	35	35	12 mo postdx	63 µg	Yes
	Gender: Female	Race: White						
8600	Yes	Ovary	Yes	35	35	12 mo postdx	63 µg	Yes
	Gender: Female	Race: White						
1100	Yes	Ovary	Yes	35	35	12 mo postdx	63 µg	Yes
	Gender: Female	Race: White						
1500	Yes	Ovary	Yes	35	35	12 mo postdx	63 µg	Yes
	Gender: Female	Race: White						
1325	Yes	Ovary	Yes	35	35	12 mo postdx	63 µg	Yes
	Gender: Female	Race: Black or African-American						

[1]Either name of cancer site or ICD9 code of cancer site is returned.
[2]1st Degree relatives only.
[3]Returns amount remaining if available from site, otherwise amount stored is returned.

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Internet

Generating Statistical Methodologies and Computational Tools

Vital to successful biomarker discovery are analytic approaches that enable thorough, yet robust, analysis of massive databases generated by modern biotechnologies, such as microarrays for genetic markers and time-of-flight mass spectrometry (TOF-MS) for proteomic markers.

The Network's Data Management and Coordinating Center is developing such approaches for protein biomarker discovery in collaboration with the Biomarkers Development Laboratory at Eastern Virginia Medical School. The principles and methods developed are not limited to one type of data, but easily extend to other proteomic and genomic data. Key developments are summarized below.

Pre-analysis Data Processing

Prior to a statistical analysis of marker discovery, TOF-MS data requires pre-analysis processing to extract relevant information, standardize the data, and summarize the data. Based on eminent properties of the data, pre-analysis processing first identifies all protein signals that are distinguishable from noise, then calibrates mass (per charge) values of proteins for potential measurement errors, and finally aggregates, as a single signal, multiple protein signals that are within the range of measurement errors. The Data Management and Coordinating Center continues to modify this algorithm to improve performance.

Disease Classification

Collaborating researchers are developing algorithms to use SELDI serum protein fingerprints to classify prostate cancer, benign prostate hypertrophy (BPH), and normal state, based on differential serum protein patterns. The rationale is to directly analyze the whole spectrum of data for disease classification without protein identification. Because the protein expression profile for each sample consists of thousands of measurements (mass/charge and intensity), conventional statistical methods are not able to analyze such high dimensional data. Data dimension reduction was achieved using discrete wavelet transformation in discriminant analysis.

Wavelet is a method used in computer science for signal compression, but had not been used for proteomic data analysis. This method is useful for disease classification using data generated from SELDI if no peak identification and normalization algorithm is available. The wavelet data is then analyzed by the Kullack-Leibler statistical discrimination measure, to identify the wavelet coefficients that will form the classifier. This biostatistical classification algorithm yielded a sensitivity of 97% and specificity of 100% in detecting prostate cancer in a study set consisting of 386 serum samples (96 age-matched normal men, 92 BPH, and 197 prostate cancer).

Protein Biomarker Identification

Though wavelet transformation gives excellent classification, back transforming wavelet coefficients to identify proteins important for classification is difficult. When peaks are identified by the pre-processing analysis and the dimension is reduced, it is then more desirable to use an analytical method that can lead to protein identification as well as good classification of diseases. To achieve both good classification and interpretation, a modification of a boosting method, originally developed in the 1990s by computer scientists, was used to develop a decision tree classifier for the diagnosis of prostate cancer from the non-cancer controls.

The key feature of boosting is the ability to select the signals in a robust manner but that avoid overfitting (i.e., selecting noise as signal), which is a major challenge in analyzing high dimensional data. Boosting first starts with a number of single protein mass peaks, each of them usually classifies poorly. However, by iteratively reweighting the importance of each promising protein mass peak, a combination of these single protein mass peaks can achieve a much better classifier.

Using the same serum study set described for the biostatistical classification algorithm analysis, a single decision tree classifier was developed that was 90% accurate yielding a sensitivity of 83%, a specificity of 97%, and a positive predictive value of 96% in differentiating prostate cancer from the non-cancer controls using 9 protein masses.

The boosting-logistic regression decision tree classifier used 21 protein peaks to distinguish normal vs. abnormal samples (i.e., prostate cancer and benign prostate hypertrophy) with 96.67% accuracy. A nearest neighbor classifier using 14 peaks selected by the boosted decision tree method has 100% specificity and 93.33% sensitivity for detecting prostate cancer.

Currently, Data Management and Coordinating Center investigators are extending this methodology of boosting learning to the situation in which the disease status of a sample is subject to error—a common situation in biomedical settings. For example, in the analysis of the prostate cancer data there is a 20%-30% possibility for a patient with a diagnosis of BPH related to multiple negative biopsies, to harbor cancer cells, thereby potentially resulting in a false negative classification.

Artificial Intelligence Learning Algorithms

Besides the biostatistical wavelet classification, decision tree, and boosting classifiers developed by the Data Management and Coordinating Center, the Eastern Virginia Medical School laboratory has evaluated other artificial intelligence learning algorithms to process the SELDI and develop a diagnostic classifier.

A summary of the results to separate prostate cancer from the non-cancer controls follows:

Algorithm	Sensitivity	Specificity	Error Rate
BMCA (TOF data)	80%	93%	13%
Boosting BMCA* (TOF data)	97%	93%	5%
BSCA* (TOF data)	77%	90%	17%
SVM (SELDI processed data)	90%	93%	7%
Ciphergen Biosystems Pattern Matching Software: CART (SELDI processed data)	90%	97%	5%
Decision Tree* (SELDI processed data)	83%	97%	10%
Boosting Decision Tree* (SELDI processed data)	97%	97%	3%

* Developed at DMCC

BMCA=Binary Markers Combination Approach

BSCA= Biostatistical Classification Algorithm

SVM=Surface Vector Machine

CART=Classification and Regression Trees

The best accuracy in differentiating the prostate cancer from the benign and normal controls was with the two-decision tree and boosting algorithms. However, these results suggest that it will be important to evaluate SELDI profiling data sets by more than one bioinformatics tool in an effort to find the diagnostic classifier that accurately separates the cancer population from the non-cancer controls without over-fitting the data. Another important observation in these studies is that accurate data analysis depends entirely on having an efficient and reproducible peak selection and alignment algorithm with biostatistical confidence.

Genomic and Proteomic Data Mining

Data are rapidly accumulating from genomic and proteomic analyses. A key concern of Network statisticians is how to develop algorithms to combine information from multiple biomarkers to identify preclinical cancer. When gene expression profiles or protein mass spectrometer profiles are the basis for identification, the number of potential markers (genes or proteins) is enormous. Innovative analytic tools are needed to provide the best metric for biomarker evaluation.

The design and evaluation of exploratory studies is perhaps the most promising and difficult statistical issue in biomarker development. Consider the analysis of gene expression array data and the problem of ranking genes in regards to their differential expression in cases and controls.

Classical statistical measures, such as the t-statistic, do not necessarily identify the most useful genes.

The Data Management and Coordinating Center is proposing several novel approaches to disease screening, including one that has been successfully applied to an ovarian cancer data set involving 30 cases and 23 controls. Researchers have developed a strategy for sample size calculations for phase 1 studies that is based on having a high probability of identifying the most differentially expressed genes. Again this is different from and more appropriate than traditional sample size calculations that are based on statistical hypothesis tests.

System Screening

One of the important potential uses of markers for early cancer detection is to monitor target populations via large public screening programs. It is crucial to explore and understand factors that influence the accuracy (sensitivity and specificity) of such programs.

Evaluating the accuracy of a single diagnostic test has gained much attention in the statistical and applied literature, but little attention has been paid to factors affecting accuracy characteristics of a sequence of tests, as would be used in routine screening. With repeated marker measurements over time in a target population, true cases and false positives will be identified cumulatively, at each time of testing. In practice, when a single marker is used repeatedly in routine screening, the same screening threshold is typically used to define positivity at each screening visit. For instance, when *PSA* is used to screen for prostate cancer, a common threshold is 4 ng/ml.

One possible alternative is to adjust the threshold at successive visits according to individual-specific characteristics. By application to a prostate cancer screening setting using *PSA*, the Data Management and Coordinating Center has illustrated how such ideas may guide choices in designing a screening program. In the setting considered, it has been shown that adaptive thresholds may perform better than constant thresholds (such as 4 ng/ml) and that screening biannually has accuracy characteristics almost as good as annual screens, with very little difference between annual and biannual screening designs in the mean time prior to clinical diagnosis at which cases are identified.

Standardization of Reagents and Assays

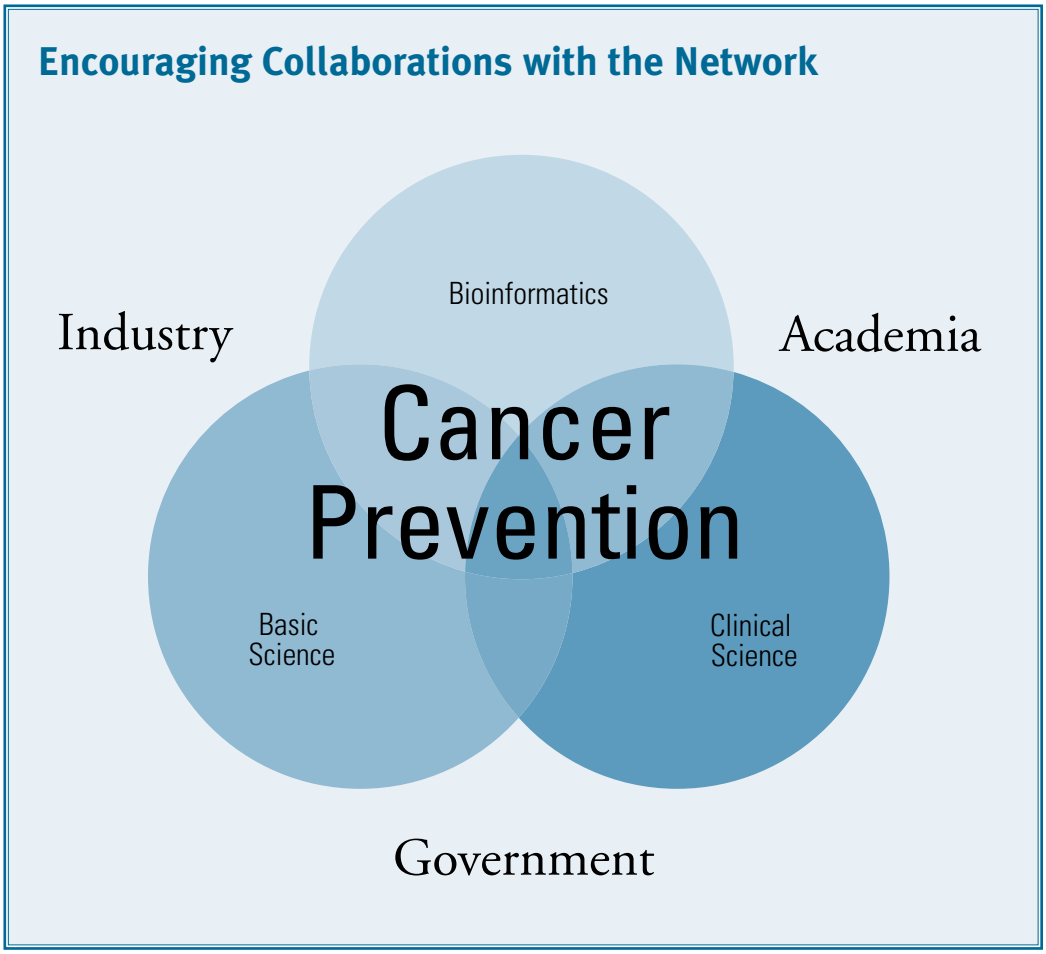
Accurate early detection screening tests must be accompanied by high-throughput assays/technologies that are reproducible and affordable. In collaboration with NIST, Network investigators continue to standardize methodologies, refine assays, and establish standard reference materials for biochemical, molecular and cytologic assays.

As described earlier, the scoring criteria and image standards for measuring chromosomal breakage as a measure of susceptibility for lung and upper respiratory tract cancers are being developed at NIST. In addition, reference threshold concentrations for mtDNA below which mtDNA cannot be measured and sequenced are being established for mutational analysis in the D-loop of mtDNA, which is known to be mutated in early stages of lung, head and neck, and bladder cancer (described on page 32).

Encouraging Collaborations with the Network

The forum provided by the Network encourages interaction and collaboration among leading cancer researchers around the world. Investigators and liaisons meet regularly to discuss how to better decipher the molecular circuitry of cells and to apply this understanding to the earlier detection of cancer. Two primary collaborative activities take place: sharing research materials, including specimens, and sharing and exchanging data and research results.

Using both the unconventional approach to biomarker research through scientific consortia, and facilitating the regular research-intensive interaction of leading experts from multiple disciplines, the Network is pursing biomarker discovery, development and validation at an unprecedented pace for both common and rare cancers.



At the heart of cancer prevention research is the overlap of efforts in bioinformatics, basic science, and clinical science by collaborating professionals in government, industry, and academia.

Associate Memberships

Collaborations between Network investigators and investigators from other U.S. and foreign institutes and industries are supported through the Associate Membership program.

Associate Members are non-Network investigators who propose collaborative studies within the scope and objectives of the Network, and often contribute by sharing available technologies, specimens, high-risk registries and cohorts, and other resources. Applications are accepted three times per year for studies that could expand resources within the Network or enable investigators to collect data to support applications for future independent funding.

Investigators planning to become Associate Members can join one of the Network's four Collaborative Groups according to their expertise and interest. The group's chairs are Network principal investigators who serve as the primary contact for nonaffiliated investigators. Interested researchers can also contact any Network principal investigator about collaboration.

The Collaborative Groups and chairs are:

- Breast and Gynecologic Cancer, Daniel Cramer, M.D., Brigham and Women's Hospital
- Gastrointestinal and Other Cancers, Dean E. Brenner, M.D., University of Michigan
- Lung and Upper Aerodigestive Tract Cancer, Mel Tockman, M.D., H. Lee Moffitt Cancer Center
- Prostate and Other Urologic Cancers, Ian Thompson, M.D., University of Texas Health Science Center

The Associate Membership program is soliciting proposals to study the following cancers: breast, mesothelioma, nasopharyngeal carcinoma, gastric cancer, pancreatic cancer, liver cancer, esophageal cancer, endometrial cancer, kidney cancers, germ cell cancers of the testis, and melanoma.

In the past three years, NCI has funded 12 awards to nonaffiliated investigators for studies that span the collaborative groups, including the activities listed here.

Breast and gynecologic cancer collaborative groups are evaluating human *kallikrein* 6 (hk6) as a biomarker for the early detection of epithelial ovarian cancer. The research screens the pre- and post-operative sera of over 400 women diagnosed with stage I ovarian cancer matched against controls using immunoassays to this candidate biomarker. The results are being compared against serum cancer antigen 125 (*CA125*) levels from the same patients.

In another effort, researchers are developing molecular markers for cervical carcinogenesis for use in translational research. The overall goal is to develop a molecular assay to be performed on routinely obtained Pap smears that will predict the risk of aggressive cervical dysplasias that may rapidly progress to invasive cervical cancer. Aberrantly methylated genes present in squamous cell and adenocarcinomas of the cervix are profiled assessing the methylator phenotype and key methylated genes in high-grade cervical intraepithelial lesions as a predictor of recurrent or persistent cervical dysplasia. This study will analyze associations between the methylator phenotype and endogenous or exogenous epidemiological risk factors.

New insights on rare tumors are emerging. An associate investigator at Wayne State University is studying development and progression of malignant pleural mesothelioma (MPM) by: (1) gene profiling for prognostication in mesothelioma, (2) methylation studies in mesothelioma and relation to SV40, and (3) SELDI analyses of mesothelioma pleural effusions. The gene array data have revealed obvious differences in gene expression of PIG3, Cell adhesion kinase B, and natriuretic receptor A between four short-term survivors and 10 long-term survivors. For the methylation studies, they have developed a novel demethylating assay in which DAC was added to SV40 infected and transformed mesothelial cells. These data have revealed that HMG1 and MIC1 are methylated in these transformants, and have been verified by re-expression verification assays using HMG antibodies. Finally, in a comparison of 30 pleural effusions to 28 control effusions, SELDI protein chip proteomics has revealed three biomarkers, which are 100% specific and sensitive for mesothelioma. Validation studies are in progress.

Novel technologies amenable to high throughput assay are under development by investigators from Van Andel Research Institute. The research is aimed at an antibody array to identify markers in the serum or pancreatic juice that would lead to the development of more sensitive, specific and minimally invasive diagnostic tests for pancreatic cancer. Microarrays comprised of antibodies to putative markers, cancer-related genes, cytokines and serum markers from other cancers are being tested to measure proteins in the sera and pancreatic juice of patients. Bioinformatic analyses of protein microarray data are being done initially through hierarchical clustering between arrays from sera of cancer patients as compared to control. These results are assessed to determine how well their clusters perform against software-derived groupings in observed samples as compared to random permutations.

Lung and upper aerodigestive tract cancers studies are testing the hypothesis that newly identified genetic polymorphisms in the *Epoxide Hydrolase* (EH) gene play an important role in risk for tobacco-related cancers. Network investigators provided more than 100 DNA samples from lung cancer and case controls. Samples from both African-American and Caucasians using DNA samples isolated from buccal specimens are being screened by PCR and single-stranded conformational polymorphism analysis of nine exons of the *EH* gene.

Associate Members

Member/Institution	Research Focus	Network Sponsor
Brian B. Haab, Ph.D. Van Andel Research Institute Grand Rapids, MI	Antibody Array Pancreas	Jose Costa, M.D.
Leonard S. Marks, M.D. Department of Urology Urological Sciences Research Foundation, Culver City, CA	*PSA* Isoforms Prostate	Alan Partin, M.D., Ph.D.
Mai H. Nguyen, M.D. University of California-Los Angeles Los Angeles, CA	DNA Permutations in Urine Breast	David Chia, Ph.D.
Carolyn Y. Muller, M.D. University of Texas Southwestern Dallas, TX	Methylated Genes Cervix	Adi Gazdar, M.D.
Jong Y. Park, Dr.P.H. Cancer Control H. Lee Moffitt Cancer Center Tampa, FL	Mutations in Epoxide Hydrolase Lung	Melvin Tockman, M.D., Ph.D.
Harvey I. Pass, M.D. Wayne State University Detroit, MI	Mesothelioma DNA Array Lung	Adi Gazdar, M.D.
Karen Smith-McCune, M.D., Ph.D. University of California School of Medicine San Francisco, CA	In situ hybridization Cervix	Elizabeth Unger, M.D., Ph.D.
Ying-Hsiu Su, Ph.D. Thomas Jefferson University Doylestown, PA	Permutations in DNA from Urine Liver, Colon, Rectum	Timothy Block, Ph.D.
Eleftherios P. Diamandis, M.D., Ph.D. Mount Sinai Hospital University of Toronto Toronto, Canada	Human Kallikreins Ovary	Sudhir Srivastava, Ph.D., M.P.H.
Holger Hebestreit Oxford Glycobiology Institute Oxford, UK	Proteomics Liver	Timothy Block, Ph.D.
Edward F. Patz, M.D. Duke University Medical Center Durham, NC	MALDI-TOF Lung Proteins	Jeffrey Marks, Ph.D.
William Grady, M.D. Vanderbilt University Nashville, TN	Methylated Genes Colon	Dean Brenner, M.D. and Henry Lynch, M.D.

Shared Technology and Resources

The Network has initiated several collaborative projects with other NCI-supported programs, such as the Cancer Genetics Network and Specialized Programs for Research Excellence, to better understand biological risk factors for familial and hereditary cancers. Accomplishments resulting from active collaborations follow.

Tissue Microarrays

The UCLA Validation Laboratory (the UCLA Defined Tumor-Marker Evaluation Core, or D-TEC) has constructed high-density tissue microarrays for the Network. Two microarrays have been completed, prostate and breast cancer, and one is under construction for lung cancer.

Tissue microarray technology is used to analyze several hundred tumor samples, as well as control/normal tissue from the same organ, on a single slide. This approach allows high throughput analysis of genes and proteins on a large cohort. The method consists of sampling core tissues from paraffin-embedded tissue donor blocks and placing them into a single paraffin block. In spite of the low amount of tissue analyzed by tissue microarray, several independent studies have demonstrated a high concordance of protein expression between this technique and the conventional tissue sections.

Implementation of high-throughput genetic technologies, such as cDNA and oligonucleotide microarrays, generates myriad points of data. The identified cancer-associated candidate genes need to be further characterized and selected using a large number of well-characterized tumors and stringent criteria. The high-throughput expression profiling of tumor samples provides key information at the micro-anatomical level.

The samples used to construct each tissue microarray are from the vast archives of the UCLA Medical Center. Each tissue-specific microarray has more than 1,000 individual spots representing approximately 500 patients. Samples are chosen as to represent all stages and grades of tumor progression, and to include different subsets of tissue-specific tumors. Matched histologically normal samples are also included. A relational database is currently being constructed to link pathology and clinical information, images of each spot, and the corresponding gene expression data.

In addition, the UCLA D-TEC program in conjunction with the Tissue Array Core Facility (UCLA Department of Pathology and Laboratory Medicine) and the Biostatistics Core (UCLA Department of Human Genetics) is optimizing conditions to construct, stain, and analyze these high-density tissue microarrays.

High Risk Cohorts

One of the early charges of the Network was to expand identification of high-risk healthy populations based on genetic predispositions and the development of new molecular markers. Advancements related to cancers of the head and neck, lung, prostate, ovary, breast, gastrointestinal, and hereditary cancer syndromes are highlighted below.

Head and Neck and Lung Cancers

University of Texas MD Anderson Cancer Center investigators in collaboration with several organizations established two collections of cohorts that mark an important resource for developing and validating new biomarkers, and identifying subgroups that can be enrolled into intensive screening programs for early detection and cancer prevention.

The first collection contains more than 1,300 cases of patients with stage I and stage II upper digestive tract cancers. Subjects were at least 18 years of age and free of cancer for up to three years after primary treatment. A second collection includes 1,100 lung cancer patients equally divided between non-smokers and smokers. The epidemiological profiles for both cohorts were established. The control pool includes more than 700 individuals matched to the cohort by gender, age (within 5 years), ethnicity and smoking status. Specimens (serum, blood lymphocytes and paraffin blocks) are available.

Prostate Cancer

Based on the knowledge that African-American men develop prostate cancer earlier in their lives and are more likely to die from it than white, non-Hispanic men, the University of Texas at San Antonio Medical Center in collaboration with the Audie Murphy VA Hospital, Brooke Army Medical Center and Wilford Hall Medical Center is conducting a prospective study of a multiethnic populations in San Antonio to determine the use of novel biomarkers in prostate cancer.

Known as the San Antonio Center of Biomarkers of Risk for Prostate Cancer (SABOR), the program will enroll 10,000-patients, including a large number of minority patients (Mexican Americans and African Americans). The registry contains 1,457 individuals of various races and ethnicity. Efforts are aimed at increasing recruitment of ethnic minorities and under-served populations. A comprehensive database established for patients participating in the study includes information about:

- Past medical history
- Current use of medications for prostate disease
- Current use of phytotherapy
- Family history of neoplastic disease
- Diet
- Ethnicity
- Anthromorphometry
- AUA urinary symptom score
- Digital rectal examination findings
- Blood pressure
- *PSA*

The repository of biological materials from SABOR features: serum, blood lymphocytes and toenails from 362 individuals; 309 frozen prostatectomy specimens; and 1,590 paraffin embedded specimens of biopsies, trans-urethral resection of the prostate and prostatectomies.

A separate group of investigators at Johns Hopkins University is accessing two different cohorts for prostate cancer studies. The first is through collaboration with the Baltimore Longitudinal Study of Aging. This cohort was established in 1958 and contains 1,500 men within the age range of prostate cancer. There is a complete epidemiological profile for each of the participants and serum samples. The second cohort of about 3,000 individuals is a result of the early detection screening program at the university's departments of Urology and Clinical Chemistry/Pathology.

Hereditary Cancers

Creighton University has developed a registry of individuals at high risk for specific cancers who are willing to participate in biomarkers studies, known as the Early Detection Research Network High Risk Registry. The registry includes 157 individuals who are carriers of germ line mutations for hereditary cancer syndromes such as hereditary non-polyposis colorectal cancer (HNPCC), hereditary breast/ovarian Cancer (HBOC), familial adenomatus polyposis (FAP), hereditary hematologic cancers, familial pancreatic cancer and others. Recruitment is ongoing.

Creighton may also recruit participants for specific Network studies from their Hereditary Cancer Family registry. This registry contains 439 colon cancer families, of which 129 families are HNPCC, 83 are with HNPCC-associated mutation, 22 families had FAP, and 4 families had *APC* gene mutations. Another 114 families were FAMMM/pancreatic cancer families, of which eight have the *p16* gene mutation. Of 933 breast/ovarian families, 92 have mutation in *BRCA 1/2* genes.

Ovarian Cancer

One of the Network's Clinical and Epidemiology Center's, the Brigham and Women's Hospital, Harvard Medical School, is collaborating with Dr. Ian Jacobs in London to collect serum from women previously enrolled in prospective studies of screening for ovarian cancer. They have collected more than 1,000 sera. The Center maintains a repository of pre-operative serum and plasma from 160 women with ovarian cancer, 300 women with benign gynecologic diseases, and 238 normal women selected from the general population.

Breast and GI Cancer

Duke/Abbott Developmental Laboratory has enrolled in a high-risk registry 220 breast patients and 100 GI patients. For the past year, more than 500 blood samples were collected with CPT tubes and white blood cell fractions were carefully cryopreserved for biomarkers recovery. All clinical information on these subjects is recorded and maintained in a password-protected database. The laboratory initiated a new protocol to collect and analyze nipple aspirates (NAF) and blood from women who are being seen at the high-risk breast clinic. NAF will be analyzed on SELDI or MALDI platforms for detection of premalignant disease. Most women entered into the study will have known *BRCA1* and *BRCA2* mutational status.

Forging Partnerships with Private Sectors

NCI anticipated from the outset that Network members would collaborate with industry both in developing biomarkers and/or reagents and providing a clinical environment for the evaluation of new technologies. It is hoped that validated biomarkers ultimately will be commercialized into diagnostic products for early detection and risk assessment.

Collaborations involving companies help the research community explore possibilities for new products and approaches, and permit private sectors to realize the value of their research investments. When such interactions occur early, they foster coordinated research plans that can benefit all partners.

However, concern about confidential information, ownership of and access to intellectual property and data that emanate from research can constrain studies. NCI's Technology Transfer Branch and Division of Cancer Prevention's Cancer Biomarkers Research Group drafted industry collaboration guidelines after the Network's Technology Resources Sharing Committee articulated a need to alleviate potential barriers to collaboration.

Feedback provided by Network members during the February 2002 Steering Committee meeting will be incorporated into final guidelines to be presented for adoption at a future meeting. Final guidelines will cover sharing reagents, biological materials, data and technology in Network-related studies between investigators and pharmaceutical and biotechnology companies. Issues relating to publication, confidential treatment of proprietary information, intellectual property rights, and licensing will also be addressed.

The guidelines recognize the rights and obligations of grantee institutions under the Bayh Dole Act (35 U.S.C. 200 et seq.) and are founded on the National Institutes of Health Grants Policy Statement, which include "Principles and Guidelines for Recipients of NIH Research Grants and Contracts on Obtaining and Disseminating Biomedical Research Resources."

Private collaborating organizations have included: Abbott Pharmaceuticals, Advanced Biosciences, Ataigin Technologies Inc., Bayer Pharmaceuticals, Bristol Myers, Circon Corp., Ciphergen Biosystems, Clenomics, COBE BCT, Inc., Corixa, Gene Logic, Genetica Inc., ORCA Biosciences Inc., Oxford GlycoSciences, Proteome Sciences, Roche, Research Genetics Inc., Spectral Imaging, Urosciences Group, and Zeneca Diagnostics. Recent endeavors are described below.

Michigan-Bayer Collaboration

The Great Lakes New England (GLNE) Clinical Epidemiology Center launched a collaboration with Bayer, which, with its subsidiary, Chiron, Inc., has made major scientific and financial commitment to discovery, validation, and commercialization of surrogate endpoint biomarkers for early cancer diagnosis. Bayer employs proteomics, genomics, and nanotechnology to discover promising new biomarkers. Teams of molecular biologists, physicists, and statisticians use state-of-the-art informatics to mine human genomic and proteomic data commercially procured or discovered in house to identify promising biomarkers. Once identified, banks of tissue samples and matching sera have been used to provide preliminary decision analysis data.

In collaboration with Network investigators and the NCI Technology Transfer Branch, a single confidentiality agreement was executed by Bayer and the five GLNE institutions. This agreement allows GLNE to develop and execute validation projects for early diagnostic and carcinogenesis biomarkers of products derived from Bayer research under Network sponsorship.

Duke University-Abbott Diagnostic Laboratory Collaboration

This research aims to identify breast-specific markers that will be useful for early detection and monitoring of disease progression. By utilizing proprietary, large expression databases from Abbott Diagnostic Laboratory, investigators at Duke University have found two new genes (*BU101* and *BS106*) that appear to be expressed primarily in breast cells (both benign and malignant). Since breast cells are not found in the blood of healthy women, the presence of these gene products may signal a growth abnormality in the breast, most likely cancer or early pre-cancerous changes. Investigators developed a series of reagents and assays for the two new genes with the goal of detecting very low levels in the blood.

This joint effort is a model for other academic-industrial partnerships in many ways. Abbott continues to support the research at Duke through transfer of reagents, instrumentation, and most importantly, intellectual property. Duke scientists have in turn, taken several of the lead markers identified by Abbott to the next stage of clinical development. Further, markers identified at Duke have been tested, in silico, using Abbott resources. At the same time, Abbott scientists have gained a position in the Network that allows them to keep abreast of current academic developments in biomarker research, contribute to the practical aspects of the science from its base of resources and experience, and possibly negotiate early licensing arrangements to facilitate rapid development of new markers.

Network-Ciphergen Collaboration

The Eastern Virginia Medical School Biomarkers Development Laboratory was the first to develop a partnership with Ciphergen, and introduced the SELDI technology to the Network as a new and innovative technology to not only discover novel cancer biomarkers but to use SELDI protein profiles to discriminate cancer from non-cancer controls. The laboratory provides training in SELDI use to Network and non-Network investigators. A number of Network laboratories are actively collaborating with Ciphergen for proteomic analysis.

Both the investigators and the company benefited from this collaboration. Investigators received priority services from the equipment maker in terms of delivery, training and other enhancements. In return, the company benefited from verification of the SELDI accuracy, precision, and sensitivity for serum and other body fluids analyses. Precision testing and calibration of the SELDI technology and molecular assays with SRMs provided by NIST will significantly further development of diagnostic reagents and tools. Partnerships with a number of biotechnology and diagnostic companies augment the precision and capacity of SELDI technology for sequencing, enhancing the ability to mine data on protein and genomic expression profiles, and increasing the accuracy of diagnostic assays.

Partnership with Standard Reference Laboratories and Other Organizations

This governmental-industrial-university relationship enhances the scientific opportunities for the Network by bringing the resources of major industrial sponsors to the collaborating institutions through mutually beneficial scientific collaboration. Over the next year, prospective cross sectional and longitudinal validation projects of promising biomarkers discovered by the Network and industrial partners will begin.

Network investigators, working with the Food and Drug Administration, plan to organize a meeting to solicit input from the World Health Organization, the National Committee for Clinical Laboratory Standards (NCCLS), the National Institute for Biological Standards and Controls (NIBSC), the Red Cross, and NIST
on the needs and requirements for a proteomics standard like NIST SRM1951a. These investigators are reaching out to a wider scientific community, including such groups as the Human Proteomic Organization, to gain the participation of researchers across the world in the effort to direct proteomics standards.

Con Edison Partnership

Con Edison, a utility firm serving the New York area and part of the Northeastern states, collaborates with the Lung Cancer Biomarker Center, a Network Clinical and epidemiological Center, under the leadership of Dr. William Rom. With the Utility Worker's Union, they are recruiting smokers for a lung cancer screening project. They have an NIH Certificate of Confidentiality for participating workers where aggregate data without any personal identifiers is supplied to Con Edison and the Utility Workers Union.

This group represents an important cohort for lung cancer biomarkers validation studies because of their potential exposure to asbestos and other substances. Con Edison has had a contract for a collaborative relationship about asbestos exposed workers since 1990 with the New York University Division of Pulmonary and Critical Care Medicine. The utility has approximately 10,000 full-time employees and 2,000 retirees, who have worked in power plants using oil or natural gas throughout the New York City region, and serviced power substations and the steam and power conduits underground and above ground in the New York Region. Workers are exposed to asbestos in the power plants where insulation is used in boilers and hot water pipes. Asbestos, solvents, lead, and other exposures can also occur in manholes and other worksites.

Dissemination and Diffusion of Research Results

Along with its primary mission to support research on cancer detection, prevention and treatment is NCI's support of the subsequent dissemination of findings.

Published abstracts are one of the best testaments to the Early Detection Research Network's collaborative approach. These documents show substantial barriers being overcome and relationships being forged. They demonstrate the continued pursuit of a better understanding of cancer cells and a Network well poised to find the missing pieces of the puzzle.

Several communications means convey the progress in biomarker research to the lay public and scientists. These include Web sites, sponsoring meetings, workshops, conferences, and participating in other national and international meetings. At the Network's first and second scientific workshops in September 2000 and October 2001, investigators presented research findings that detailed the latest advances and emerging issues in biomarker research. The two-day meeting addressed topics such as advances in organ specific molecular detection; correlative clinical studies; computational analysis and statistics; informatics; emerging issues; technology for biomarkers of risk; and technologies within the Network. More than a dozen invited speakers and over 60 abstracts fostered discussions on these and other issues. The abstracts were presented in the journal *Disease Markers*. (See Appendix I).

A highlight of EDRN's dissemination efforts was the initiation of the March 2002 scientific meeting as one of the prestigious Gordon Research Conferences. By choosing to elevate *New Frontiers in Cancer Detection and Diagnosis* to a Gordon conference, the stature of the meeting was greatly increased and it was promoted as an international forum for idea exchange and scientific discussion.

EDRN Investigators Disseminate Information Through Liaisons

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): L. Austin Doyle, M.D.,

American Society of Preventive Oncology (ASPO): Margaret Spitz, M.D., M.P.H.

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

Cancer Genetics Network: Steven Skates, Ph.D.

Cooperative Family Registries: John Baron, M.D.

Director's Challenge: 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.

Other gynecology groups (not including ACOG): David Fishman, M.D.

Pharmaceutical and industrial relations: Greg Downing. D.O.

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) (UICC): Michles Bodo, M.D.

Network Evaluation and Next Steps

The Network will continue to build on its existing resources and expertise to advance biomarkers for validation studies. Engaging scientists in translational research will occur in several ways:

- Accelerated validation of newly discovered biomarkers
- Expanded Network attention to additional organ sites for biomarker discovery and clinical validation
- Comprehensive development of genomic and proteomic fingerprints of premalignant and preinvasive lesions
- Supported translational research on epigenetic changes in identifying cancer risk, precursor lesions, and people at elevated risk
- Development of high-yield technologies for isolating exfoliated cells in body fluids, particularly for noninvasive detection of abnormal cells through, for example, seminal fluid and urine
- Using high risk cohorts for association studies between polymorphism of candidate genes and cancer risk
- Expanded public-private partnerships for validation studies by providing incentives to industry, such as access to specimen resources, while industry in return will provide necessary technological platforms
- Established international consortium on rare tumors, such as naso-pharangeal carcinoma, and mesothelioma

In short, researchers need to identify biomarkers that are predictive of clinical outcomes. Surrogate endpoint biomarkers may also provide biologic insights in the short term, and eventually provide a rationale for changes in the design of clinical trials.

Metrics to evaluate the Network's success were fleshed out by the Steering Committee. Detailed in Appendix III, these metrics define the goals of scientific excellence, productivity, and collaboration.

While the Network continues to improve, investigators will gain empowerment as the direct funding of their Centers places on them the burden of scientific leadership, research agenda, and collaboration.

At the beginning of the continuum, basic scientists with robust bench research records will be influenced to pool ideas, resources, and tools through additional core funds and supplements. Then, translational and epidemiology investigators with strong tools and publication track records will be directly funded with a mandate to translate concepts arising from the basic science labs. Concurrently, analytical tools, laboratory, statistical, and informatics activities will be directly supported with a collaborative mandate.

It is expected that leadership of this whole collaborative emanates from the grassroots investigators and the Executive Leadership will interact with this highly knowledgeable group of scientists in a manner that enhances collaboration and productivity.

Finally, the impact of new technology on reducing mortality is contingent on development of highly predictive biomarkers for earlier detection and risk assessment. The success of this effort relies in large measure on exploring the concordance between genetic or molecular markers and the morphologic changes associated with premalignant and preinvasive lesions that have life threatening potential.

Overall, the Early Detection Research Network represents a new paradigm of cooperative research that will fuel the future clinical studies on which progress in prevention will ultimately depend.

Appendix

I Key Publications by Early Detection Research Network Investigators

Adam BL, Vlahou A, Semmes OJ, Wright GL Jr. Proteomic approaches to biomarker discovery in prostate and bladder cancers. *Proteomics*. 2001; 1:1264-70.

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III Metrics for Programmatic Evaluation

Excerpted from the Manual of Operations Of the Early Detection Research Network

April 27, 2001

It is the responsibility of the awarding agency, in this case the National Cancer Institute (NCI), National Institutes of Health, to review progress achieved towards scientific goals in original grant applications over specified grant periods and to provide scientific and logistical input to grantees to enhance the quality of their scientific efforts. For details, see HHS 45 CFR, Part 74. To review progress towards achieving the objectives of the EDRN and its investigators, EDRN program officials are charged as part of the terms of the award to gather information on the functioning of the network in order to update the NCI leadership. This document describes the prospectively developed metrics, rationale, and standards for evaluating the overall success of the EDRN.

Introduction

Fair, rigorous peer review of investigator-initiated scientific applications remains the cornerstone of scientific progress in the United States. Peer review has ensured that the best science is supported. The EDRN was initiated with this concept in mind. By selecting scientific collaborators for the EDRN on the basis of rigorous peer review and fully funding the best applications, the NCI has successfully obtained strong participation from the scientific community.

The EDRN represents a major pioneering effort in collaborative translational research. It departs from prior Cancer Cooperative Group models in many important ways—through empowering investigators by funding their Centers directly and by placing the burden of scientific leadership, research agenda, and collaboration upon these directly funded Centers. Basic scientists with robust bench research records have been funded to pool their ideas, resources, and tools. Translational and epidemiological investigators with strong tools and publication track records are directly funded with a mandate to translate concepts arising from basic science labs. Analytical tools, laboratories, statistical methods, and informatics are also supported directly with a collaborative mandate. Leadership of this collaborative must emanate from the grass-root investigators, and the Executive Leadership must communicate with a highly knowledgeable group of scientists in a manner that enhances collaboration and productivity. This Network represents a new paradigm of Cooperative research.

NCI Charge to the EDRN

At the opening meeting of all of the funded EDRN units, NCI leadership and Program Staff provided the following charge for this collaborative enterprise:

- Establish criteria for the discovery and validation of biomarkers at all points of the integrated research scheme;
- Establish a rigorous quality assurance/quality control program for biomarkers;

- Establish and deal with issues of biorepositories—how the samples will be obtained, stored and most importantly, allocated;
- Support Translational Research Projects—both within and outside the EDRN—and establish policies and procedures that are inclusive of investigators who wish to utilize the infrastructure and facilities of the EDRN;
- Establish and foster industrial collaborations which will be crucial to the ability to rapidly translate the research effort into products and to test innovative biomarkers being developed by industry;
- Establish and maintain effective and efficient communications, including the use of EDRN Web sites (public and private), email, and regularly scheduled meetings;
- Develop and maintain an effective, efficient, and productive management domain with minimal committee structure and maximal collaboration, with financial rewards for collaboration;
- Encourage inclusiveness by ensuring that scientists with promising research ideas get the opportunity to collaborate productively with the EDRN.

Evaluation Metrics

Since there are no prior models of such a cooperative research enterprise devoted to early detection biomarkers, it is very important to carefully monitor and assess progress from both macro and micro perspectives. This review will be particularly important during the first grant period in which substantial administrative effort should be expended in order to build the new infrastructure. The following evaluation metrics are to be used:

For the Individual Laboratory and Center:

1. Scientific Excellence

Quality of Questions: Has the EDRN site clearly defined their objectives, hypotheses, and scientific plan? Scientific Progress to Date: Has the EDRN site made progress towards meeting these objectives as specified in their originally funded research plan? What pitfalls have been encountered and how have they been managed?

Innovation: How has the EDRN site used innovation to overcome obstacles? Is the site aware of new methods or approaches that might be useful to or portable into the EDRN environment?

Future Plans: What does the site plan to do over the coming two years? How will these plans meet the original grant objectives?

2. Productivity Metrics

Publication productivity: Has the site published papers on the objectives funded by the EDRN? How many and in what Journals? If not, are there problems that need to be addressed or require assistance?

Grant funding: Has the site applied for additional peer-reviewed grant or contract funding? Has the site team been successful in gaining additional funds? Has the EDRN been helpful to the success of funding these new grants or contracts?

Biomarkers identified (BDLs): Number of new biomarkers pursued for evaluation? Number of biomarkers sent forward to CECs or BVLs for validation? Number of biomarkers added to early detection or risk assessment panels? Number of biomarkers used in chemoprevention clinical trials?

Assays performed (BVLs): Numbers of assays developed for EDRN projects? Numbers of samples processed? Types of samples processed? Results reported? Quality control of samples assayed? Number and type of development projects approved? Use of CDEs?

DMCC: Standards of informatics support? Type of informatics, QC procedures, patient privacy protection measures, data storage, and retrieval systems for Validation Studies? Development of Network-wide communication systems? Development of Network-wide systems to promote data and specimen sharing? Development of statistical methodology to meet the needs of EDRN?

Samples collected and provided (CECs): Numbers of samples collected? Types of samples collected? Sources of samples collected? Numbers of samples provided to EDRN BDLs or BVLs? Use of CDEs? How many CECs have had their set-aside funds released? How many CECs have requested the release of Developmental funds?

3. Collaborative Metrics

EDRN collaborations: With whom is the EDRN site collaborating? How many projects are collaborative? How many joint papers have been published? Use of EDRN resources: Has the EDRN site collaborated with CECs, a BVL or BDL site? If so, how many? Joint publications? Joint grants? How many BDLs have requested release of their restricted funds for Network Collaborative Studies?

Participation in EDRN Activities: Attendance from the site at EDRN meetings. Participation on Committees, working groups, and task forces? Special EDRN projects completed. Did EDRN site participate in developing the CDEs? Did EDRN site help to standardize/streamline the IRB approval process? Did EDRN site help develop systems for streamlining data sharing and/or specimen sharing? Did EDRN site help develop systems to standardize/streamline technology transfer issues?

EDRN outreach: Number of new Associate Members from the outside? Amount of Chair's funds allocated to new Associate Members? The number of applications for Chair's funding? Other outreach activities?

Process for Evaluating Metrics:

1. Annual written progress report

Reviews should be based upon the yearly progress report required for non-competitive renewal. Instructions for preparation of the non-competitive renewal should be specific and emphasize progress towards scientific goals of the original grant application and progress towards addressing EDRN's mission. While scientific quality and progress need to be recorded and addressed, primarily, metrics should be required to allow NCI staff to report data to NCI leadership.

The review process should assess the progress of each of the funded units towards meeting the specific aims of their funded grant application and their progress and contributions in meeting the above-described charges for the entire group. While the review is structured to provide NCI leadership and staff with data to track the progress of the EDRN and its components, equally important goals are to provide constructive feedback to EDRN Principal Investigators and their collaborators. Reviews may be used by EDRN leadership, NCI staff, and the Network Consulting Committee to make mid-course changes or to encourage constructive changes in individual scientific direction or focus. Initial reviews might assist in building collaborations among investigators and their groups. Reviews may also be used to assess administrative progress, to quantify publications and grants, and to quantify numbers of subjects studied.

2. Site Visits

Each Center/Laboratory should be site visited by a panel comprised of external consultants (individual members of the Network Consulting Committee), NCI staff and other experts on an as-needed basis. The site visit should be brief (preferably a half day or less) but enable a thorough review of scientific progress, future scientific plans, performance metrics, facilities and staff in support of the EDRN charge. The site Principal Investigator would provide a 2-3 hour presentation period to review scientific progress, spell out new scientific initiatives for EDRN research, and address required metrics. The Principal Investigator should be encouraged to share problems, concerns, and questions to the site visit team so that the process is interactive and collegial. While an agenda and presentation should be necessary, no scoring should be used.

3. Frequency of Site Visits

The frequency of the site visits will be determined by the NCI. However, it is anticipated that one initial site visit by NCI program officials, in year one will occur, and one mid-grant site visit (for a five-year grant, it will be between the year 2 and year 3). Additional site visits may be required when deemed necessary by the NCI.

Deficient performance and remedies will be conducted in accordance with HHS 45 CFR, Part 74 and other pertinent regulations.

Overall Evaluation of Early Detection Research Network:

It is the intention of the NCI that the members of the Network Consulting Committee and Chairs and Co-Chairs of the EDRN Steering Committee will discuss the overall performance of the EDRN using the metrics presented in this document and suggest changes/modifications in the working structure of EDRN for the next five-year cycle.

Cancer Center Review Model: Perhaps the best model to evaluate the success of the EDRN is a Cancer Center type review. View the EDRN as a "Cancer Center" that is devoted to discovery and translation of surrogate endpoints for early cancer detection and risk assessment. In a Cancer Center review, the Center's leadership and Program Directors are asked to demonstrate how the sum exceeds the total of its parts. This approach requires all program leaders, participating investigators, and Center leadership to demonstrate how the Core facilities, administration of resources, and Core grant funding have enhanced scientific progress. This review measures collaborative efficacy.

IV Institutional Reveiw Board Guiding Documents

Excerpted from Manual of Operations of the Early Detection Research Network

M E M O R A N D U M (draft)

Date:

To: [Name of Institutional IRB Chairs & Administrators]

From: [Name of EDRN investigator]

Subject: Early Detection Research Network (EDRN) proposed IRB standardized review

This memo is to discuss with you the possibility of setting up a standardized IRB review system for the Early Detection Research Network (EDRN).

The EDRN is an NCI-sponsored, peer-reviewed, Institutional Review Board (IRB) approved consortium of 31 investigator-initiated research projects organized to develop and validate biomarkers of risk and early detection of human cancer. Collaborations between EDRN investigators and investigators from the United States, private industries, and foreign institutes are encouraged. Associate Membership, as this collaboration is called, can be sought through an established EDRN investigator and approved through the EDRN Steering Committee. EDRN collaboration and validation studies may include as few as two institutions or extend to many EDRN institutions. EDRN research studies will involve sharing of biological samples, data, and in some cases reviewing medical records.

The following EDRN objectives include human subject research activities requiring IRB review:

- develop and test promising biomarkers or technologies
- evaluate promising, analytically proven biomarkers or technologies
- collaborate with academic and industrial leaders in molecular biology, molecular genetics, clinical oncology, computer science, public health and clinical application for early cancer detection
- collaborate and rapidly disseminate information among EDRN members and associate members

The NCI sponsored an EDRN IRB working group to provide a forum where EDRN human subject issues could be discussed on a regular basis and common IRB concerns could be addressed and resolved. This group is comprised of six EDRN investigators and their IRB representatives (chairs and/or institutional office Administrator) from across the country, NCI representatives, and George Gasparis from the Office of Human Research Protections (OHRP). Of specific interest to this group was finding a way to streamline the IRB review process over the multiple EDRN sites, while maintaining the high level of protection of our human subjects.

This letter is to propose setting up a standardized EDRN IRB review process with EDRN local IRBs. This would consist of using a standardized IRB applications form and a consent form template that will be modified according to each collaborative project and each local IRB requirements. We have discussed this with George Gasparis (OHRP) who endorses the concept of an IRB standardized review process.

Since January, 2001 this IRB working group has been working on developing these forms, which are attached for your review. By using these forms, we assure continuity in addressing questions that are specific to EDRN and also present EDRN collaborative study proposals to the IRBs in a standardized format for ease of IRB review. We welcome your feedback to these forms.

After you have had an opportunity to review this material, I welcome an opportunity to discuss this concept with you further and answer any questions you might have. Because I was not part of the EDRN IRB working group, some of your questions may best be answered by those who were involved in the process. Please feel free to contact Cim Edelstein, Study Administrator of the EDRN Data Management and Coordinating Center at 206-667-4995; e-mail address cedelste@fhcrc.org, or Karen Hansen, Director of the Fred Hutchinson Cancer Research Center Institutional Review Board, at 206-667-4867; e-mail address khansen@fhcrc.org. They have both worked on developing similar systems with other large multi-centered trials and can provide you with detailed information.

Thank you in advance for considering this proposal. We believe that it will work well for both EDRN and with the local EDRN IRBs.

IRB Cover Sheet and Final Application

The goal of the application process is to ensure that the Institutional Review Board (IRB) is provided with sufficient documentation to determine that research studies are ethically sound. It is the intent of the application form to guide the investigator to areas that must be addressed when designing the protocol in order to maximize the protection of subjects.

Date:_____ Protocol #:_____

EDRN Site:_____

Shaded Area for Review Office Only

IR#:_____ Date Rec'd:_____

Title of Protocol/Activity: _____

Principal Investigator: _____

Institution: _____

Address: _____

Phone: _____ E-mail:_____

Other Investigators: _____

Type of Review Requested:

❑ New IRB Protocol

❑ Full Review ❑ Expedited Review

❑ Cooperative Review ❑ Review of Exempt Status

❑ Revision to previously application, IR# _____

Funding Agency: _____

Title of funding proposal: _____

(if different than IRB study title)

Dates of Funding: from_____ to_____

Approvals:

Local EDRN Approvals:

_____ Principal Investigator (type)	Date of EDRN scientific review:_____ _____ Division /Department Head *(type)*
_____ Signature Date	_____ Signature Date

Final Approvals:

_____ Name (type)	_____ Signature
_____ Title	_____ Date
Dates of approval_____	to_____

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17 Checklist – Order of Attachments

1 Summary/Outline of Activity

1.1 Summarize the background and rationale for this activity.

1.2 Describe the objectives which are to be met.

2 Research Participant Profile

2.1 Summarize the criteria for selection and/or exclusion of participants.

2.2 What are approximate number and ages of the following?

	NUMBER OF PARTICIPANTS Entire Study	AGE RANGE OF PARTICIPANTS Entire Study
PARTICIPANTS/CASES		
NORMAL/CONTROLS		
OTHERS (specify)		

2.3 Will participant population include equitable gender representation?

❑ Yes ❑ No

a. If no, please explain:

2.4 Will participant population include equitable minority representation?

❑ Yes ❑ No

a. If no, please explain:

3 Populations Requiring Special Considerations

3.1 Will illiterate or non-English speaking populations be included in the research?
❑ Yes ❑ No

a. *If yes*, please describe the procedures to be used to ensure there is informed consent in this population:

3.2 Will this study involve prisoners as research subjects?
❑ Yes ❑ No

a. *If yes*, please use the following definitions to check the involvement of the research participant for each category of research in the table below: (Check all that apply.)

Category 1 – A study of the possible causes, effects, and processes of incarceration, and of criminal behavior, provided that the study presents no more than minimal risk and no more than inconvenience to the subjects. [45CFR46.306(a)(2)(A)]

Category 2 – A study of prisons as institutional structures or of prisoners as incarcerated persons, provided that the study presents no more than minimal risk and no more than inconvenience to the subjects. [46CFR46.306(a)(2)(B)]

Category 3 – Research on conditions particularly affecting prisoners as a class, provided that the study may proceed only after the Secretary has consulted with appropriate experts including experts in penology, medicine, and ethics, and published notice, in the Federal Register, of his intent to approve such research. [45CFR46.306(a)(2)(C)]

Category 4 – Research on practices, both innovative and accepted, which have the intent and reasonable probability of improving the health or well-being of the subject. In cases in which those studies require the assignment of prisoners in a manner consistent with protocols approved by the IRB to control groups which may not benefit from the research, the study many proceed only after ... [45CFR46.407]

Research with prisoners	**Cases**	**Controls**	**Other**	**None of these**
Category 1 (study of incarceration, minimal risk)	❑	❑	❑	❑
Category 2 (study of institutional structures, minimal risk)	❑	❑	❑	❑
Category 3 (research on conditions affecting prisoners)	❑	❑	❑	❑
Category 4 (research on practices with intent of improving health of subject)	❑	❑	❑	❑

3.3 Will this study involve minors (as defined by state law) as research subjects?
❑ Yes ❑ No

a. *If yes*, please use the following definitions to check the involvement of the research participant for each category of research in the table below: (Check all that apply.)

Category 1 – Research not involving greater than minimal risk. [45CFR46.404]

Category 2 – Research involving greater than minimal risk, but presenting the prospect of direct benefit to the individual subject. [46CFR46.405]

Category 3 – Research involving greater than minimal risk with no prospect of direct benefit to individual subjects, but likely to yield generalizable knowledge about the subject's disorder or condition. [45CFR46.406]

Category 4 – Research not otherwise approvable, but which presents an opportunity to understand, prevent, or alleviate a serious problem affecting the health or welfare of children. [45CFR46.407]

Research with minors	Cases	Controls	Other	None of these
Category 1 (not greater than minimal risk)	❑	❑	❑	❑
Category 2 (greater risk, but with prospect of direct benefit)	❑	❑	❑	❑
Category 3 (greater risk, with no prospect of direct benefit)	❑	❑	❑	❑
Category 4 (not otherwise approvable)	❑	❑	❑	❑

3.4 Will this study involve pregnant women as research subjects?
❑ Yes ❑ No

a. *If yes*, please use the following definitions to check the involvement of the research participant for each category of research in the table below: (Check all that apply.)

Category 1 – The purpose of the activity is to meet the health needs of the mother and the fetus will be placed at risk only to the minimum extent necessary to meet such needs. [45CFR46.207(a)(1)]

Category 2 – The risk to the fetus is minimal. [46CFR46.207(a)(2)]

Research with pregnant women	Cases	Controls	Other	None of these
Category 1 (minimum necessary risk to fetus)	❑	❑	❑	❑
Category 2 (minimal risk to fetus)	❑	❑	❑	❑

3.5 Will this study involve women of childbearing potential as research subjects? [If yes, consent requirements found at 45CFR46.116(b)(1)]
❑ Yes ❑ No

3.6 Will this study involve people not competent to provide informed consent? [If yes, 45CRF46111(b) states additional safeguards need to be taken as a criteria for IRB approval. Additional guidance may also be found at the NIH web site: http://grants/nih/gov/grants/policy/questionablecapacity.htm]
❑ Yes ❑ No

4 Recruitment

4.1 Will you obtain names and addresses of individuals for potential study recruitment from a registry or repository containing confidential information?
❑ Yes ❑ No

a. *If yes*, what is the source?

b. Provide a letter of support from the gatekeeper of the registry or repository authorizing your access to the identifiable data for the purposes of your study been obtained?

4.2 When, how, and by whom will the participants be recruited?

4.3 (Check all that apply.)

❑ Advertisements
❑ Brochures
❑ Contact letters
❑ Flyers
❑ Internet (other than Physicians' Data Query)
❑ Radio
❑ Television
❑ Other (describe)_____
❑ None of these
_____

5 Informed Consent

5.1 When, and by whom, will the participant or participant's legal guardian initially be approached for consent?

5.2 Where will informed consent be obtained (e.g., in a clinic, investigator's private office, participant's home, etc.)?

5.3 If nurses, CRAs, social workers, etc., will be involved in the informed consent process, indicate what they will do and their roles in the process.

5.4 In addition to the consent form, what method(s), if any, will be used to educate the potential research participant of the research project and their rights as a participant? (Check all that apply.)

❑ Brochures
❑ Conference with participant and family member(s)
❑ Conference with interpreter
❑ Follow-up discussion
❑ Video
❑ Other (describe)

5.5 What method(s), if any, will be used to evaluate the understanding of the potential research participant of the research project and their rights as a participant? (Check all that apply.)

❑ Pre- and post-test
❑ Verbal feedback
❑ Other (describe)

5.6 What type(s) of informed consent will be used? (Check all that apply.)

❑ Written consent signed by the participant and/or the participant's legal guardian [45CFR46.116(a)]
❑ Oral consent statements or written study overview not requiring documented signature by the participant or participant's legal guardian [45CRF46.117(c)]
❑ Waiver of consent [45CRF46.116(d) and 45CFR164.512]

6 Confidentiality/Autonomy

PLEASE NOTE:
Federal certificates of confidentiality may be issued for studies that could expose subjects and their families to adverse economic, psychological and social consequences were an investigator compelled to disclose identifying information in any civil, criminal, administrative, legislative or other proceeding whether federal, state, or local. Contact the Office for Human Research Protection for additional guidance about obtaining a certificate of confidentiality.

6.1 Describe the steps taken to assure that the identities of participants will be kept confidential. What safeguards are used to protect against identifying, directly or indirectly, any participant in any report of the research project?

6.2 Will data with participant identification be available to any of the following?

❑ Study staff ❑ IRB

❑ NIH/NCI ❑ FDA

❑ Drug company (sponsor) ❑ Other (specify):

6.3 Is identifying information required for the purposes of another research project?

❑ Yes ❑ No

a. *If yes*, please describe this project:

7 Research Procedures

7.1 List, in sequence, a brief description of procedures that will be followed in this activity/protocol:

7.2 Will patients receive intervention or follow-up care outside of the local institution under the auspices of a non-local physician?

❑ Yes ❑ No

a. *If yes*, please explain:

7.3 Will your study involve collection and analysis of biological specimens for research purposes?

❑ Yes ❑ No

If yes, please respond to each of the following:

a. Are the results currently useful for predicting the [future] occurrence or prognosis of disease?

❑ Yes ❑ No

If yes, please explain:

b. Will the results be potentially useful for predicting the [future] occurrence or prognosis of disease?

❑ Yes ❑ No

If yes, please explain:

c. Will the results potentially be used for predicting disease risk/susceptibility in family members?

❑ Yes ❑ No

If yes, please explain:

d. Will the research test results be provided to the research participant?
❑ Yes ❑ No

If no, please explain:

If yes, confirm that tests will be performed in a clinically certifiable manner (i.e., CLIA approved laboratory), and then explain how such information will be provided to each participant and his/her health care provider, including pre- and post-test counseling, if applicable. Also describe what steps will be taken to assure access to this information does not jeopardize the participant's privacy or confidentiality and/or medical record.

❑ Yes, tests will be clinically certifiable ❑ No, tests will not be clinically certifiable

7.4 Will your study include collection/storage of biological specimens for future research analysis?
❑ Yes ❑ No

If yes, please respond to each of the following:

a. What types of specimens will be stored?

❑ Blood fractions (e.g., whole blood, serum, plasma, buffy coat, blood spots)

❑ Tumor samples

❑ Bone marrow ❑ Saliva ❑ Urine

❑ Buccal swabs ❑ Sputum ❑ Extracted DNA/RNA

❑ Establishment of permanent cell lines

❑ Other specimen, specify:

b. Can any of these specimen be used to derive DNA/RNA? ❑ Yes ❑ No

c. Does your informed consent address this future use?
❑ Yes ❑ No

If yes, be sure the consent document includes a clear description of

1) the operation of the cell repository,

2) the specific types of research to be conducted,

3) the conditions under which data and specimens will be released to recipient-investigators, and

4) procedures for protecting the privacy of subjects and maintaining the confidentiality of data.

If no, please explain:

d. If participant withdraws, what happens to the specimen and/or data?

7.5 Will HIV serostatus be evaluated or be an eligibility criteria for participants on this study?
❑ Yes ❑ No

If yes, please provide the rationale for testing and an outline of procedures to be followed:

7.6 Will your activity involve the collection of family medical history information?
❑ Yes ❑ No

If yes, please respond to each of the following:

a. Are personal identifiers (i.e., first and last name, address, phone number) collected on family members?
❑ Yes ❑ No

b. Will you have access to personal identifiers?
❑ Yes ❑ No

If no, who does have access to the identifiers?

c. How will this family history information be used?

d. Is family history information (e.g., disease status of members) shared among family members as part of this study?
❑ Yes ❑ No

If yes, does the consent form describe this activity?

If no, what steps will be taken to assure participant confidentiality (including that of the relatives) be protected?

7.7 Are there specific components of this study that will be provided free of charge to the participant?
❑ Yes ❑ No

If yes, please explain and include details in the informed consent:

7.8 Are there additional expenses related to this protocol?
❑ Yes ❑ No

If yes, please explain and include details in the informed consent:

7.9 Will participants be paid or otherwise compensated for research participation?
❑ Yes ❑ No

If yes, please respond to the following:

a. What is the amount of compensation? $_____

b. If not monetary, what will be used for compensation?

c. What is the reason for compensation?

d. Who is responsible for preparing the compensation and how is the participant's confidentiality protected?

8 Health and Safety Considerations

8.1 Will materials with potential radiation risk be used either in vitro or in vivo?

❑ Yes ❑ No

If yes, please describe type of radiation below and provide the Memorandum of Understanding (MUA) approval date and letter for each institutional site:

8.2 Will this study involve the use of recombinant DNA?

❑ Yes ❑ No

If yes, please provide EMUA approval date and letter for each institutional site:

8.3 Will this study involve use of a select carcinogen?

❑ Yes ❑ No

If yes, please provide CMUA approval date and letter for each institutional site:

8.4 Will this study involve direct gene transfer?

❑ Yes ❑ No

If yes, please provide approval date and letter for each institutional site:

9 Risk vs. Benefit Analysis

9.1 Summarize the nature and amount of risk (including side effects) or substantial stress or discomfort involved. Examples of risk include physical risks, psychological risks (such as substantial stress, discomfort, or invasion of privacy), and social risks (such as jeopardy to insurability or employability).

9.2 Summarize the overall plans for minimizing risks.

9.3 Describe the potential benefits of the research for the individual subjects.

9.4 Describe the potential benefits of the knowledge gained to others.

9.5 Explain how the potential benefits of the research outweigh the potential risks and how this is acceptable.

10 Alternatives

10.1 If the study involves clinical interventions, please identify alternative procedures, if any, not proposed for this study that might be advantageous to the participant.

11 Investigational New Drugs

11.1 Does this study involve an Investigational New Drug (IND)?

❑ Yes ❑ No

If yes, please provide the following:

a. Drug name_____

b. IND#_____

c. Holder of IND_____

d. FDA Status (o I, o II, o III, or o IV)

e. Dosage_____

f. Responsible PI at each institutional site:_____

_____

_____

_____

12 Investigational Devices

12.1 Does this study involve an Investigational Device (IDE)?

❑ Yes ❑ No

If yes, please provide the following:

a. Device name_____

b. IDE#_____

c. Holder of IDE_____

d. Responsible PI at each institutional site:_____

_____

_____

_____

12.2 Is this a significant risk or non-significant risk device?

❑ Significant risk device ❑ Non-significant risk device

13 Monoclonal Antibodies and Biologics

13.1 If monoclonal antibodies and/or biologics are use, which ones?

14 Conflict of Interest

14.1 Does the investigator or any other person responsible for the design, conduct, or reporting of this research have an economic interest in or act as an officer or director of any outside entity whose financial interest would reasonably appear to be affected by this research?

❑ Yes ❑ No

If yes, please answer the following:

a. Has the investigator's involvement with the outside entity been approved by institutional compliance offices?

❑ Yes ❑ No

b. If the economic interest is significant, has a plan for eliminating or managing any conflict been approved?

❑ Yes ❑ No

If yes, please describe the plan and attach a copy of the approval:

15 Local EDRN Member Institution Requirements

15.1 Use this section to identify state laws, local policies, and requirements of your local IRB (for example, 1572 forms, genetic testing laws, health care information laws).

16 Investigator's Statement

As Principal Investigator, I acknowledge:

a That I am responsible for reporting any emergent problems, any adverse effects or reactions, or proposed procedural modifications and that no modifications will be put into effect without prior Institutional Review Board (IRB) approval except where necessary to eliminate apparent immediate hazards;

b) That unless otherwise directed by the IRB Chairperson, I will renew this application with the IRB at least annually;

c) That the research project is being conducted in compliance with the IRB's understanding and recommendations;

d) That the IRB is provided all the information on the research project necessary for its complete review; and

e) That this research project will not be put into effect until final IRB approval is received.

Signature of Principal Investigator Date

17 Checklist – Order of Attachments

17.1 Protocol/Activity Plan and Grant Application

❑ Yes ❑ N/A

If N/A, please provide an explanation below:

17.2 Letters of Approach/Recruitment

❑ Yes ❑ N/A

If yes, list below in sequential order:

17.3 Consent Forms

❑ Yes ❑ N/A

If yes, list below in sequential order:

17.4 Questionnaires

❑ Yes ❑ N/A

If yes, list below in sequential order:

17.5 List of performance sites and/or off-site investigators participating in this protocol

❑ Yes ❑ N/A

If yes, list below in sequential order:

17.6 Review and Approval through the Recombinant DNA Advisory Committee (RAC)

❑ Yes ❑ N/A

If yes, please provide a copy of the completed "Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols" prepared for the RAC:

17.7 Relevant Health and Safety Office's Approval Letters, including radiation safety and biologic safety letters

❑ Yes ❑ N/A

If yes, list below in sequential order:

17.8 Investigational New Drugs Investigator's Brochure, drug booklet, or information sheet supplied by the drug company (sponsor)

❑ Yes ❑ N/A

17.9 Other pertinent information

❑ Yes ❑ N/A

If yes, list below in sequential order:

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