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<b>13. ABSTRACT (Maximum 200 Words)</b>  The goals of the Advanced Cancer Detection Center include the discovery of molecular and genetic markers of cancer risk, the identification of individuals at high risk for cancer through screening and the testing of methods to prevent cancer. The projects included in this report are:  <div style="margin-left: 40px;">                     Cad Vs. Human Accuracy in the Interpretation of Screening Mammograms: A Pilot Study                       Lung Cancer Screening with Computed Tomography: Initial Results of Cohort Screening Trial                       The Tampa Bay Ovarian Cancer Study                       Development of the Moffitt Cancer Network                 </div>
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## **INTRODUCTION:**

The **Advanced Cancer Detection Center** (ACDC) of the H. Lee Moffitt Cancer Center and Research Institute at the University of South Florida received initial funding in October 1997. In 2001, funding that was appropriated in FY00 and FY01 was awarded separately to the University of South Florida for the project period 2001-2006. This new award was made because several projects funded from the original award were still ongoing and funds in the original award were obligated to complete them. Those projects included:

*Epoxide Hydrolase Genetic Polymorphisms and Their Functional Significance,*

*Automated Quantified Screening for Melanoma,*

*Breast Cancer Screening in High-Risk Women: Comparison of Magnetic Resonance Imaging (MRI) with Mammography, and*

*Adaptive Computer Assisted Diagnosis (CAD) Method for Lung Nodule Early Detection.*

and were reported in the final report of DAMD17-98-1-8659. One project, *Development of the Moffitt Cancer Network*, continues beyond the earlier DoD grant and is included in annual progress reports for the current award, DAMD17-01-2-0056. As new projects are funded under the new initiative, their progress reports will also be included.

The ACDC has addressed the goals identified in its appropriations language through studies that target the discovery of molecular and genetic markers of cancer risk, the identification of individuals at high risk for cancer through screening, and the testing of methods to prevent cancer. In addition, the ACDC created a technology base that provides online video streaming, video supported web casting and teleconferencing and the development and application of expert systems. The success of these efforts has led to advances in cancer detection (publications) and the development of systems that have attracted additional peer-reviewed funding.

In order to accomplish the overall programmatic goals, the Advanced Cancer Detection Center supports research and demonstration projects that further its mission. Preference is given to projects that extend the system's development and have the potential to lead to independent peer reviewed funding. During the current grant period, the ACDC supported two cancer prevention and control research protocols, both of which were funded under the first Advanced Cancer Detection Award and their funding continued under this award. For this reason they are included in this progress report as well as in the final report of the previous award. The supported studies are:

Cad Vs. Human Accuracy in the Interpretation of Screening Mammograms: A Pilot Study (C Beam, PhD and W. Qian, PhD)

Lung Cancer Screening with Computed Tomography:Initial Results of Cohort Screening Trial (Robert A. Clark, M.D., Todd Hazelton, M.D., Lynn Coppage, M.D., Thomas N. Chirikos, Ph.D., Frank Walsh, M.D., Mark Rolfe, M.D., Lary Robinson, M.D., Eric Sommers, M.D., Nina R. Wadhwa, M.S.P.H., Gerold Bepler, M.D., Jeffrey Krischer, Ph.D., Melvyn Tockman, M.D., Ph.D).

**BODY:**

Overview: The H. Lee Cancer Moffitt Center & Research Institute includes a free standing patient care facility with a large inpatient and outpatient capacity, a major research institute consisting of more than 130 scientific members, a free standing Lifetime Cancer Screening Center and a wide array of outreach and educational activities for the general public and select underserved populations. Moffitt Cancer Center's location at the convergence of the University of South Florida's Health Sciences Center and the main campus sets the stage for its conceptual commitment to interdisciplinary approaches to research and patient care. Moreover, it allows the Center to enjoy all intellectual advantages of a matrix center while remaining operationally freestanding. After 18 years, the Cancer Center's mission remains totally focused on "contributing to the prevention and cure of cancer."

The Cancer Center was created by the Florida Legislature in the early 1980s, to meet a clear and compelling need to respond to Florida's "cancer epidemic." Building a major cancer research and treatment center at the University of South Florida in Tampa was largely the vision of H. Lee Moffitt, a state legislator who served as Speaker of the Florida House of Representatives from 1982-84. Construction of the original, 380,000 square foot hospital facility was funded with \$70 million from the state's cigarette tax, allowing the Center to open in 1986.

The initial phase of the Cancer Center's strategic plan called for a rapid and substantial deployment of its clinical, financial, and philanthropic resources to develop a true scientific center of excellence. The Center recruited Dr. John C. Ruckdeschel as the Cancer Center's first director in late 1991. In 1992, he began fulfilling that strategic plan, a process that culminated in the awarding of a Cancer Center Support Grant (CCSG) five years later.

The strategic plan's second phase continues the focus on scientific and clinical growth, with a commitment to increase research facilities by over 200,000 sq.ft., and to prepare to accommodate twice as many patients by 2009. In 1998, the state legislature committed an additional \$100 million to finance the construction needed to meet these goals.

In August, 2002, Dr. William Dalton was recruited to become the Cancer Center Director replacing Dr. Jack Ruckdeschel. Dr. Dalton was the Dean of the College of Medicine at the University of Arizona and previously was the Associate Center Director for Clinical Investigations at the Moffitt Cancer Center for 5 years. Thus, Dr. Dalton brings to his new role a considerable experience in the operations of the Cancer Center and an in-depth background in the development of the Cancer Center's scientific agenda.

In April, 2003, Dr. Krischer stepped down as program leader for the Cancer Control Program and returned to the faculty to focus on research. Dr. Thomas Sellers was recruited from Mayo Clinic to be the Associate Center Director for Cancer Control and the new program leader.

Today, the Cancer Center's membership numbers 150 scientists and clinicians who are USF faculty. More than 94 members-in-residence are housed and supported in the Center's facilities and work under the terms of the USF/Moffitt affiliation and faculty support agreements. Other members are based in University departments. The Cancer Center's 1,500 employees support the work of the physicians and scientists. The Center has annual operating revenues of over \$130 million yearly, including an \$11 million annual appropriation from the State of Florida, research grants totaling more than \$36 million overall (direct), philanthropic donations, and institutional commitment from the University of South Florida in the form of faculty salaries and a portion of clinical practice revenues.

The Cancer Center currently supports four scientific programs:

<u>Program</u>	<u>Leader</u>	<u>Members</u>
Molecular Oncology	Richard Jove, Ph.D.	21
Immunology	Julie Djeu, Ph.D.	14
Clinical Investigations	Timothy Yeatman, M.D.	58
Cancer Control	Thomas Sellers, Ph.D.	39
Non-aligned members & institutional grants	N/A	5

The Cancer Control Program consists of two subprograms: cancer prevention and health outcomes and behavior. Dr. Krischer's research activities are programmatically aligned with the health outcomes and behavior subprogram. A number of faculty are active collaborators in Dr. Krischer's program. They include:

- Dr. Dmitry Goldgof, Associate Professor, Computer Science and Engineering, College of Engineering
- Dr. Pamela Munster, Assistant Professor, Department of Interdisciplinary Oncology, College of Medicine
- Dr. Rebecca Sutphen, Associate Professor, Department of Interdisciplinary Oncology, College of Medicine
- Dr. Nagi Kumar, Associate Professor, Department of Interdisciplinary Oncology, College of Medicine
- Dr. Paul Jacobsen, Professor, Department of Psychology, College of Arts and Sciences
- Dr. Jennifer Mayer, Associate Professor, Department of Pediatrics, College of Medicine
- Dr. Larry Hall, Professor, Computer Science and Engineering, College of Engineering
- Dr. Cynthia Myers, Assistant Professor, Department of Interdisciplinary Oncology, College of Medicine

Dr. Rachel Richesson, Assistant Professor, Department of Pediatrics, College of Medicine

These faculty members participate in ACDC projects or contribute to other research initiatives of Dr. Krischer's group with funding from multiple peer-reviewed sources. The successful competition for these funds has permitted the development of multiple research studies and a technology advanced infrastructure that supports them.

The funding of the Advanced Cancer Detection Center is one of three mechanisms by which this has occurred.

### **Advanced Cancer Detection Center**

The Advanced Cancer Detection Center has become a significant component of the infrastructure in that it provides a stimulus for research development and promotes inter and intra programmatic collaborations. The Advanced Cancer Detection Center supports pilot studies that can lead to peer-reviewed extramural funding. Projects supported by this mechanism follow a two-tiered scientific review process in which the science and the likelihood of peer-reviewed extramural funding are considered. In addition, priority is given to projects that foster inter and intra-programmatic collaborations.

Recognizing the great success of this effort, the focus of the Advanced Cancer Detection Center has worked to complement the other infrastructure mechanisms in most notably the Community Clinical Oncology Program Research Base (described below). That program also provides funds for pilot studies. This has led to the consolidation of the internal advisory committee for each program so that there is continuity between programs. The membership of the consolidated internal advisory committee includes some members from the existing Advanced Cancer Detection Center advisory committee as well as leaders of the Community Clinical Oncology Program Research Base. For 2003-04, the members are:

Dr. Pamela Munster, Assistant Professor, Department of Interdisciplinary Oncology, College of Medicine

Dr. Nagi Kumar, Associate Professor, Department of Interdisciplinary Oncology, College of Medicine

Dr. Rebecca Sutphen, Associate Professor, Department of Interdisciplinary Oncology, College of Medicine

Dr. Jennifer Mayer, Assistant Professor, Department of Pediatrics, College of Medicine

Dr. Paul Jacobsen, Professor, Department of Interdisciplinary Oncology, College of Medicine and Department of Psychology, College of Arts and Sciences

Dr. Jeffrey Krischer, ex officio, Professor, Department of Interdisciplinary Oncology, College of Medicine

These members reflect expertise in genetics, nutrition, behavioral science, endocrinology, oncology, pediatrics and epidemiology. Some have been the principal investigators of studies that have previously received Advanced Cancer Detection Center support and all have experience in obtaining peer-reviewed research support.

**Moffitt CCOP Research Base (PI:Krischer)**

The H. Lee Moffitt Cancer Center received funding by the NCI in June 2000 to develop a research base as a mechanism for Community Clinical Oncology Programs to access cancer control clinical trials. NCI CCOPs and Moffitt affiliates are eligible to participate in the Moffitt CCOP Research Base. Membership is based on continued funding as an NCI CCOP with satisfactory performance measured by accrual and data quality.

The goals of the Moffitt CCOP Research Base are to:

- Develop cancer control trials of high scientific merit for implementation in the community setting.
- Provide community investigators an opportunity to participate in NCI-supported cancer control clinical trials.

The following CCOPs have, or are in the process of, establishing formal affiliations with the Moffitt CCOP research base:

Florida Pediatric CCOP, Tampa, FL  
Merit Care Hospital CCOP, Fargo, ND  
Mount Sinai Medical Center CCOP, Miami, FL  
South Texas Pediatric MBCCOP, San Antonio, TX  
Baptist Center Research Institute CCOP, Memphis, TN  
Cancer Research for the Ozarks CCOP, Springfield, MO  
Columbus CCOP, Columbus, OH  
Greater Phoenix CCOP, Phoenix, AZ  
North Shore University Hospital CCOP, Manhasset, NY  
NorthWest CCOP, Boise, ID  
Southern Nevada Cancer Research Foundation CCOP, Las Vegas, NV

The Moffitt CCOP Research Base is now staffed and cancer control protocols and concepts are being initiated. Several of the clinical studies are the result of pilot development funded by ACDC projects. All are approved by the internal advisory committee and then reviewed and approved by the National Cancer Center before activation. Examples of current studies are:

The Specific Role of Isoflavones in Reducing Prostate Cancer Risk	Protocol
A Randomized Pilot Clinical Trial of the Action of Isoflavones and Lycopene in Localized Prostate Cancer: Administration Prior to Radical Prostatectomy.	Protocol
The Effect of Cyproheptadine (peractin) and Megestrol Acetate (Megace) on Weight in Children with Cancer/Treatment Related Cachexia	Protocol
Cancer Genetic Counseling and Testing by Telemedicine Concept in Community Settings Adderall-XR Versus Concerta for Cancer	Protocol

Treatment-Related Neurocognitive Sequellae and Depression in Pediatric Patients: A Randomized Phase II Study.

Stress Management Training for Patients Undergoing Radiotherapy Protocol

Oral Glutamic Acid to Decrease Vincristine Toxicity in Children with Cancer Concept

Preservation of Ovarian Function in Young Women Treated with Neoadjuvant Chemotherapy for Breast Cancer: A Randomized Trial Using the GnRH Agonist (Triptorelin) During Adjuvant Chemotherapy Protocol

### **Data and Technology Coordinating Center, Rare Diseases Clinical Research Network**

To address the challenges inherent in diagnosing and treating rare diseases, the National Institutes of Health (NIH) created the Rare Diseases Clinical Research Network. With \$51 million in grant funding over five years from several NIH components, the network will consist of ten Rare Diseases Clinical Research Centers (RDCRCs) and a Data and Technology Coordinating Center (DTCC).

The RDCRCs and the DTCC are to be located at the following institutions:

-- Baylor College of Medicine, Houston, TX - Rare Disease Clinical Research Center for New Therapies and New Diagnostics - Dr. Arthur L. Beaudet

-- Boston University School of Medicine, Boston, MA - Vasculitis Clinical Research Network - Dr. Peter A. Merkel

-- Children's Hospital Medical Center, Cincinnati, OH - Rare Lung Diseases Clinical Research Network - Dr. Bruce C. Trapnell

-- Children's National Medical Center, Washington, DC - Rare Diseases Clinical Research Center for Urea Cycle Disorders - Dr. Mark L. Batshaw

-- The Cleveland Clinic Foundation, Cleveland, OH - Bone Marrow Failure Clinical Research Center - Dr. Jaroslaw P. Maciejewski

-- University of Rochester, Rochester, NY - Nervous System Channelopathies Pathogenesis and Treatment - Dr. Robert C. Griggs

-- The Mount Sinai School of Medicine, New York, NY - The Natural History of Rare Genetic Steroid Disorders - Dr. Maria I. New

-- University of Colorado Health Sciences Center, Denver Colorado - Cholestatic Liver Disease Consortium - Dr. Ronald Sokol

-- University of North Carolina, Chapel Hill, North Carolina - Genetic Diseases of Mucociliary Clearance Consortium - Dr. Michael Knowles

-- Duke University - Rare Thrombotic Disease Clinical Research Consortium - Dr. Thomas Ortel

-- University of South Florida and the H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL - The Data and Technology Coordinating Center - Dr. Jeffrey P. Krischer

Approximately 25 million people in the United States are affected by an estimated 6,000 rare diseases or conditions. Diseases to be studied in the centers include: urea cycle disorders; Angelman's syndrome; Prader-Willi syndrome; Rett syndrome; periodic paralysis; non-dystrophic myotonic disorders; episodic ataxia; aplastic anemia; paroxysmal nocturnal hemoglobinuria; single lineage cytopenias, including granular lymphocyte leukemia, pure red cell aplasia, and myelodysplastic syndromes; vasculitis disorders; inborn defects in steroid hormone pathways; alpha-1 antitrypsin deficiency; lymphangioleiomyomatosis; pulmonary alveolar proteinosis; and hereditary idiopathic pulmonary fibrosis.

With a collaborative approach, the network will focus on identifying biomarkers for disease risk, disease severity and activity, and clinical outcome, while encouraging development of new approaches to the diagnosis, prevention, and treatment of rare diseases.

The network will facilitate increased collaboration and data sharing between investigators and patient support groups working to improve the lives of those affected by these diseases and potentially prevent or eliminate these diseases in the future.

This network supports the re-engineering of the clinical research enterprise component presented recently in the "Roadmap for Medical Research" by Dr. Zerhouni, NIH Director. Each research center consists of a consortium of clinical investigators partnering with patient support groups and institutions within and outside of the United States that have agreed to work together studying a group of rare diseases. In addition to fostering collaborative research, the RDCRCs will train new investigators for the represented rare diseases and provide content for a public Web site on rare diseases research.

Integration of various kinds of data including genetic, microarray, clinical, laboratory, and imaging, is one of the goals of this informatics approach to clinical research being pursued at the University of South Florida. The RDCRCs and their sites will work with the DTCC in developing common data elements, data standards, and data structures. The DTCC will incorporate new approaches to data sharing and federated databases at

distributed sites that are scaleable or have the potential for future expansion and adaptation. This approach will enable researchers to integrate data with other clinical networks such as the National Electronic Clinical Trials and Research (NECTAR) network.

Each RDCRC will utilize the resources available at the General Clinical Research Centers -- 82 facilities distributed across the United States that provide clinical investigators with specialized research environments and specially trained research personnel. Supported by NCRR, the facilities include nursing staff, research subject advocates, and various core technologies, including sophisticated laboratories, nutrition staff, and imaging facilities.

The Moffitt Cancer Center and Research Institute is one of the clinical sites of the RDCRN through its affiliation with the Bone Marrow Failure Consortium, based at Cleveland Clinic and through its close association with the Data Technology and Coordinating Center.

Drs. Rachel Richesson, Larry Hall and Jeffrey Krischer all receive support from this funding mechanism which further enhances the technology infrastructure that has been built.

In fiscal year 2005, the Advanced Cancer Detection Center will further develop its Telemedicine and Informatics initiatives as a means to further its education objectives contained in enabling legislation. Those technologies already developed as part of the ongoing Moffitt Cancer Network will be expanded and further developed to achieve the following objectives:

**Task 1: Develop and implement Pediatric Internet Telemedicine Homecare study to assess efficacy of low bandwidth monitoring, management and treatment in the care of childhood chronic diseases.**

In conjunction with All Children's Hospital in St. Petersburg Florida we plan to expand the low-bandwidth video streaming capability developed under the Moffitt Cancer Network to implement a pediatric telemedicine homecare study to assess efficacy of this technology. We hypothesize the use of general monitoring and management devices can greatly improve the transfer of accurate information about the patient's condition to the physician as well as provide the physician a window inside the patient home to evaluate various complications of his or her disease. We believe the heightened amount of accurate information in addition to remote access to care will improve the ability of the physician and caregiver to care for the patient resulting in overall better care.

**Task 2: Develop and implement proof of concept study for genetic counseling delivered from a distance via telemedicine in a multi-center environment.**

In conjunction with the Florida Cancer Genetics Network (FCGN), a network of eleven sites providing genetic counseling throughout the state of Florida, we plan to implement a proof of concept study for delivering genetic counseling via telemedicine in a multi-

institutional environment. The FCGN is based at the Moffitt Cancer Center and was developed initially under Advanced Cancer Detection Center funding. The Genetics program at the Moffitt Cancer Center recently concluded a proof of concept for genetic counseling via telemedicine that showed promising results. The proof of concept was designed in such a way as to assess the technology as well as the patient and counselors resistance to or acceptance of the delivery mode. The patient and counselor were physically located in the same building, although the encounter took place via telemedicine with the use of audio and videoconferencing software.

We developed the first internet-based system for cancer genetics risk assessment, genetic counseling and research registry participation. The system automates collection of the family and personal medical history information required for these processes. Data may be 1) entered online or 2) entered on paper forms that can be faxed into a web server for direct (automated) data entry accomplished within minutes. Once entered, data is available for viewing, editing and printing via a secure website. The system generates a family pedigree and risk calculation that can also be viewed or printed from the website. For research initiatives, data in the system can easily be queried to determine the number of individuals available who meet specific eligibility requirements. Authentication and authorization features allow easy access to all data for which the user has permission, while restricting all other data from access. Web access to the system requires a standard web browser (such as Microsoft Internet Explorer version 5.5 or higher) and use of free encryption software available on the internet. The system has two main uses – 1) it automates the data collection, pedigree-drawing and risk assessment procedures of clinical genetic counseling for hereditary cancer susceptibility quickly and easily and 2) it facilitates enrollment of individuals with high cancer risk in a registry designed for individuals who are interested in participating in cancer research studies. The internet-based design of this system makes it accessible to cancer genetics centers around the world.

We propose to extend the scope of the study mentioned above to include multiple centers as well to assess efficacy using well defined tools to detect differences in knowledge transfer and patient outcomes relating to overall state of mind post counseling. This extends the current capabilities of the Cancer Network to make scarce resources more widely available to targeted populations and health care providers. In addition, we are exploring the extension of this effort to include pediatric genetics screening and counseling and have developed an extension for neurofibromatosis, which is a programmatic initiative of the DoD.

**Task 3: Develop and implement an interactive intelligent search and representation system for mining disease information to aid in proper diagnosis.**

The system that will be built is a dynamic, self-organizing network of information that will adapt to user needs. When completed, this system will model the data, use machine learning to adapt its' own search mechanisms, store its own statistics, be scalable and 100% dynamic. It will also combine web presentation technologies with analytical systems, require initial education, and be classification and utilization based. There will

be a way to add new information into the system and a way to change how the system learns.

The completed system will dynamically create web pages that display the data that the user has interest in. It will base its choices on the user's current path and statistical information about relationships or links between topics. Each user will be able to take a completely different path through the information and find completely different information in the same amount of time.

The more general statement of the problem is to semantically define relationships among granular data elements that reflect a structure imposed on the data by the user. This is equivalent to representing data in a structure such that the user can find related elements without having to know, a priori, the data structure. For example, to be successful in finding a folder that has been filed, the user might be better off knowing the filing system that determines whether the folder has been placed. The filing system might be alphabetical order, subject order, or some other ordering approach. If the filing system is organized by subject, then the user might have to know which is the most closely related subject heading for the file being sought. Yet, the user might have no awareness of how subjects are defined or even named. Similarly, if the task is to retrieve related files, then alphabetical ordering systems provide limited relational groups as compared to subject order filing systems, as long as the definition of the subject groups is explicit. Taken more generally, both data structures require the user to understand the data structure to be successful in any given query. This research will focus on more general data structures that encode relationships and do not require the user to have any prior knowledge. We will explore the application of this approach to the design and construction of web pages, in the context of the Cancer Network, although the problem is much more general.

**Task 4: Upgrade existing hardware ad server environment to replace aging equipment and maintain a state-of-the-art data and informatics infrastructure.**

The ACDC, in the coming year, will continue to replace outdated equipment as well as add new technologies that foster new research. The primary network infrastructure will consist of a gigabit switched network connected to Internet2 through redundant sonic wall firewalls. Backup and storage systems are also being upgraded. The current version of Netbackup (running on Sun Solaris) has been purchased in conjunction with a ADIC LTO2 Tape library. This will aid significantly to ensuring enterprise backups are secure and reliable. The tape library and Netbackup system will be connected to an upgraded Fiber Channel 2 Storage Area Network (SAN).

The Storage Area Network consists of redundant FC2 McData Switches and two EMC CX300 Fibrechannel arrays in a RAID 5 configuration. Each machine will connect redundantly to the SAN and be allocated space on an as needed basis. Netbackup and the tape array mentioned above are connected directly to the SAN and backups will be done directly over the high speed SAN when possible. This greatly increases our ability to adjust rapidly to surges in demand of storage so common in today's IT world.

Upgraded Oracle production and development servers have been purchased and are being installed. Upgraded versions of Oracle have been secured as well. Once installation of the new system is complete, the existing database environment will be migrated from the outdate servers to the new ones. Sun V280Rs have been purchased to house Oracle. An Oracle

Upgraded SAS production and development servers have been purchased and are being installed. The new Sun V240s will provide a significant improvement in analysis times.

Primary and Backup Domain Controllers are being upgraded to new Dell Poweredge 2650s and Window 2003. This will allow us to utilize updates to active directory and the new security measures within Windows 2003. Exchange 2003 is being implemented in concert with the upgrade of the domain controllers.

Web production, certification, and development servers are being upgraded. With the ever increase influx of .Net technologies and the subsequent integration of the technologies into Windows 2003 it is prudent to upgrade the machines and migrate to Windows 2003. The tight integration of 2003 and .Net will ease development while improving programmatic efficiency and reducing development time.

A number of additional systems are being upgraded in conjunction with the systems mentioned above. The systems being upgraded are out of date for the applications they are running and/or the applications themselves are to be updated. These include, but are not limited to, the online automated pedigree system, the Automated Patient Response system which allows phone based randomization to clinical trials, and teleforms which allows automated fax in data collection for a number of ACDC projects.

A remote site will be setup at All Childrens Hospital Pediatric genetics department when space is available and a VPN tunnel setup to ride over the existing USF-Tampa to USF-St Pete ATM network.

The infrastructure upgrade currently taking place is a critical part of the further development of the network. The network continues to be a testbed of new technologies that foster and enhance research.

#### **KEY RESEARCH ACCOMPLISHMENTS:**

The material that follows in this section summarizes the key research accomplishments associated with each project and task outlined in the appropriate approved Statement of Work for ACDC approved projects during the previous year.

Cad Vs. Human Accuracy in the Interpretation of Screening Mammograms:  
A Pilot Study

(C Beam, PhD and W. Qian, PhD)

Dr. Qian and team have successfully created and validated a CAD algorithm on an independent set of digitized mammograms selected from the "VIDI" research program (cases were acquired under R01CA74110). In addition, Dr. Qian and team have successfully applied the CAD algorithm to another set of 130 cases. These latter cases are composed of screening and diagnostic mammograms and represent breast cancer, benign breast disease and normal mammographic features. Hence, the CAD data have been collected.

Analysis of the accuracy of the CAD system compared to human observers is now underway. An initial summary of the performance of CAD with respect to callback rates in screening is provided below. If we assume that the case is called-back whenever the CAD finds either a calcification or a mass, we observe that the system has screening "sensitivity" (correctly calling back a case with cancer) of  $24/27=89\%$  and screening False Positive Probability of 29 out of 30 (97%). From the ROC perspective, a diagnostic test should always have Sensitivity exceeding False Positive-or else it performs less than that expected by chance. We observe that, at this point in analysis, the CAD performs less accurately than the toss of a fair coin.

Obviously, the CAD system is not meant to replace the screening radiologist but to assist and the previous analyses point out the need to measure the performance of the CAD against the presence or absence of calcification and mass. In preparation for that analysis, Dr. Beam and his team are registering the location of lesions on each of the cases using the original radiologist's report as the gold standard. That step was accomplished in November 2003. We then reanalyzed the performance of CAD in a manner similar to the above and compared this lesion-specific performance against that of the 110 radiologists who participated in the VIDI studies. We anticipated that analysis would be completed by the end of the year and formed the foundations of a competing continuation application submitted March 1, 2004.

### **Lung Cancer Screening with Computed Tomography: Initial Results of Cohort Screening Trial**

(Robert A. Clark, M.D., Todd Hazelton, M.D., Lynn Coppage, M.D., Thomas N. Chirikos, Ph.D., Frank Walsh, M.D., Mark Rolfe, M.D., Lary Robinson, M.D., Eric Sommers, M.D., Nina R. Wadhwa, M.S.P.H., Gerold Bepler, M.D., Jeffrey Krischer, Ph.D., Melvyn Tockman, M.D., Ph.D).

Eligible subjects were asymptomatic women and men 45 years of age or older, with a history of cigarette smoking of at least 30 pack-years. The screening study design includes one baseline screening round and four subsequent annual screening rounds. The cohort consists of 1151 enrolled subjects. Overall, 59% of subjects were male and 41% female, and the mean age was 60.2 years. The mean pack-years of smoking history were 57.9 pack-years. Baseline screening detected 28 neoplasms, and 25 cases of non-small cell lung cancer (NSCLC) (2.2% prevalence rate). Of baseline NSCLC cases, 14

were stage 1 (56%), 12 stage 1A. To date in the study, 18 incidence cancers have been detected, 15 of which are NSLC; 10 of these cases were stage 1 (66%). Overall, 60% of detected lung cancers were stage 1. Our results confirm that screening CT identifies small and early-stage lung cancers. However, small cancer size does not always correlate with early stage disease.

### **The Tampa Bay Ovarian Cancer Study**

(Rebecca Sutphen, M.D., Jeffrey Krischer, Ph. D.)

A bioactive lysolipid (LL), lysophosphatidic acid (LPA), has been proposed as a biomarker for early detection of ovarian cancer, based on results of a previous study showing elevated levels in the plasma of ovarian cancer patients compared with controls. LPA has a role in ovarian cancer proliferation, invasion and metastasis. In the present study, plasma LPA and related LL were measured in 118 case patients with ovarian cancer and 28 healthy control subjects, using a sensitive electrospray ionization/mass spectrometry method (ESI/MS). There were statistically significant differences between preoperative case samples (N=45) and control samples (N=28) in the mean levels of total LPA, total lysophosphatidylinositol (LPI), sphingosine-1-phosphate (S1P) and the following LL subspecies: 20:4-LPA, 22:6-LPA, 16:0-LPA, 18:0-LPA, 18:1-LPA, 18:2-LPA, 16:0-An-LPA, 16:0-A-LPA, 18:0-An-LPA, 18:0-A-LPA, total A-LPA, the combination of 20:4-LPA/16:0-LPA, 20:4-LPI, 16:0-LPI, 18:0-LPI, 22:6-LPC, and 18:2-LPC ( $P=.0001, .0001, .0003, .0001, .0004, .0001, .0003, .002, .03, .0001, .002, .0002, .02, .0001, .0001, .0001, .0001, .0001, .0001$  and  $.003$ , respectively). The combination of 20:4-LPA and 16:0-LPA yielded the best discrimination between preoperative case samples and control samples, with 90.4% correct classification, 91.1% sensitivity and 89.3% specificity. Total LPA achieved 89.0% correct classification, with 91.1% sensitivity and 85.7% specificity. There were also statistically significant differences between preoperative case samples (N = 45) and postoperative case samples (N=95) in mean levels of the following LL: (mean X in preoperative cases (95% CI = x - x) and mean X in postoperative cases (95% CI = x-x) ( $P < .x$ ). In 22 cases with both preoperative and postoperative samples, the postoperative levels of total LPA, S1P, total LPC, 22:6-LPA, 18:0-LPA, the combination of 20:4-LPA/22:6-LPA, 20:4-LPC and 18:2-LPC were significantly lower than preoperative levels ( $P=.03, .03, .05, .02, .04, .03, .02$ , and  $.003$ , respectively). We conclude that total LPA, total LPI, S1P and various subspecies of LPA, LPI and LPC may be useful as biomarkers of ovarian cancer. Further investigation of their use for both ovarian cancer screening and detection of recurrence is warranted.

### **Development of the Moffitt Cancer Network**

(Jeffrey Krischer, Ph.D., Dmitry Goldgof, Ph.D., Larry Hall, Ph. D.)

The technology of the Moffitt Cancer Network has been extended and implemented multiple new settings. An application has been developed for the Rare Diseases Clinical Research Network and the Community Clinical Oncology Research Base, as described above. In each application we have used the technology to implement online

teaching methods using streaming video (e.g., the CCOP Research Base) and the use of web-based video conferencing (The RDCRN). A media center has been created for each of these applications to extend our previous work and focus on making the technology more generalizable.

During the preceding year, we have begun the planning for upgrading the systems and replacing aging equipment to remain technologically current. We plan to complete the re-engineering of the network, with extensions to include the advances in telegenetics, in the coming year.

### **REPORTABLE OUTCOMES:**

- **Manuscripts, abstracts, presentations:**

Nallamshetty L, Eschrich SA, Cuthbertson D, Malloy J, Goldgof DB, Alexander AM, Trucco M, Ilonen J, Akerblom HK, Krischer JP, TRIGR Study Group: An Expert System for Evaluating Risk of Type-1 Diabetes. *In Proceedings of the 2003 IEEE International Conference on Systems, Man and Cybernetics* 1660-1665, 2003.

Sutphen R, Xu Y, Wilbanks GD, Fiorica J, Grendys EC Jr, LaPolla JP, Arango H, Hoffman MS, Martino M, Wakeley K, Griffin D, Blanco RW, Cantor AB, Xiao YJ, Krischer JP: Lysophospholipids are Potential Biomarkers of Ovarian Cancer. *Cancer Epidemiol Biomarkers Prevention* 13(7):1185-1191, 2004.

Robert A. Clark, M.D., Todd Hazelton, M.D., Lynn Coppage, M.D., Thomas N. Chirikos, Ph.D., Frank Walsh, M.D., Mark Rolfe, M.D., Lary Robinson, M.D., Eric Sommers, M.D., Nina R. Wadhwa, M.S.P.H., Gerold Bepler, M.D., Jeffrey Krischer, Ph.D., Melvyn Tockman, M.D., Ph.D. Lung Cancer Screening with Computed Tomography: initial results of a cohort screening trial, Submitted *Radiology*.

- **Patents and licenses applied for and/or issued:**

**Development of the Moffitt Cancer Network**

A notice of disclosure has been filed with the USF office of patents in anticipation of the completion of a patent application.

- **Funding received based on work supported by this award:**

The Data and Technology Coordinating Center for the NIH Rare Disease Network (PI: Jeffrey Krischer, Ph.D.)

The Data Coordinating Center for the Study of the Environmental Determinants of Diabetes in the Young. (PI: Jeffrey Krischer, Ph.D.)

**CONCLUSIONS:**

The Advanced Cancer Detection Center continues to be successful. Some projects originated under the previous funding (DAMD17-98-1-8659) have been completed under the auspices of this award and others are continuing. The research has led to publications, presentations and successful grant applications. All projects have been approved for human subjects both at the University of South Florida Institutional Review Board and at the DoD Human Subjects Review Committee.

The Advanced Cancer Detection Center has been successful in developing and implementing a variety of leading edge technologies over the past five years. We plan to continue developing new technologies as well as extending existing technologies that contributes to the improvement in quality of overall patient care and public health.

**REFERENCES:**

References pertinent to the individual projects are contained in the appended material.

# Appendices

# Lysophospholipids Are Potential Biomarkers of Ovarian Cancer

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## Abstract

**Objective:** To determine whether lysophosphatidic acid (LPA) and other lysophospholipids (LPL) are useful markers for diagnosis and/or prognosis of ovarian cancer in a controlled setting. **Method:** Plasma samples were collected from ovarian cancer patients and healthy control women in Hillsborough and Pinellas counties, Florida, and processed at the University of South Florida H. Lee Moffitt Cancer Center and Research Institute (Moffitt). Case patients with epithelial ovarian cancer ( $n = 117$ ) and healthy control subjects ( $n = 27$ ) participated in the study. Blinded LPL analysis, including 23 individual LPL species, was performed at the Cleveland Clinic Foundation using an electrospray ionization mass spectrometry-based method. LPL levels were transmitted to Moffitt, where clinical data were reviewed and statistical analyses were performed. **Results:** There were statistically significant

differences between preoperative case samples ( $n = 45$ ) and control samples ( $n = 27$ ) in the mean levels of total LPA, total lysophosphatidylinositol (LPI), sphingosine-1-phosphate (S1P), and individual LPA species as well as the combination of several LPL species. The combination of 16:0-LPA and 20:4-LPA yielded the best discrimination between preoperative case samples and control samples, with 93.1% correct classification, 91.1% sensitivity, and 96.3% specificity. In 22 cases with both preoperative and postoperative samples, the postoperative levels of several LPL, including S1P, total LPA, and lysophosphatidylcholine (LPC) levels and some individual species of LPA and LPC, were significantly different from preoperative levels. **Conclusion:** LPA, LPI, LPC, and S1P appear useful as diagnostic and prognostic biomarkers of ovarian cancer. (Cancer Epidemiol Biomarkers Prev 2004;13(7):1185-91)

## Introduction

The mortality rate for women with ovarian cancer is very high, with an estimated 14,300 deaths from ovarian cancer in 2003 in the United States (1). More than two thirds of patients have late-stage metastatic disease at initial diagnosis with a 5-year survival rate of ~20% to 30% (1-4). Conversely, at early stages, the long-term survival rate approaches 90% (5). There is currently no proven effective method for early detection of ovarian cancer through biomarkers, imaging, or other means. The most common biomarker for ovarian cancer, CA 125, lacks specificity and is elevated in only about 50% of stage I ovarian cancer cases (3, 4, 6). Proteomic patterns derived from surface-enhanced laser desorption/ionization mass spectroscopy analysis have recently shown promise for early ovarian cancer detection (7), but further

studies regarding their reproducibility and reliability for early detection and screening are needed.

Lysophosphatidic acid (LPA) has been proposed as a sensitive biomarker (8). However, studies investigating the utility of LPA as a biomarker for early detection of ovarian cancer have yielded conflicting results. Preliminary findings from a study, which included 48 healthy controls and 48 women with ovarian cancer, showed that plasma LPA levels (measured by gas chromatography) were elevated in patients with ovarian cancer ( $P < 0.001$ ; ref. 8). Importantly, elevated levels were detected in early-stage ovarian cancers compared with controls (8). The study also compared available CA 125 values with LPA levels, and results suggested that plasma LPA may be a more sensitive marker for ovarian cancer, particularly for stage I disease (8). A recent Korean study of only three pairs of samples also showed differences between ovarian cancer cases and controls (9). However, in another study where LPA levels were measured in plasma samples from 32 patients with ovarian cancer and 32 healthy controls using a liquid chromatography/mass spectroscopy assay, results showed no significant elevation in plasma LPA levels in ovarian cancer patients compared with controls, raising questions about the utility of plasma LPA levels for early detection of ovarian cancer (10).

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LPA is present in the ascitic fluid of patients with ovarian cancer (11, 12) and may function as an autocrine factor, contributing to ovarian cancer proliferation, cell survival, angiogenesis, and metastasis (13-22). Lysophosphatidylinositol (LPI), a related lysophospholipid (LPL) to LPA, has also been found at increased levels in ascites fluid and plasma of ovarian cancer patients compared with controls (23) and has been shown to display signaling properties in cellular systems (24, 25). Thus, LPI may also have utility as a biomarker of ovarian cancer, and data suggest that measuring LPI in addition to LPA may increase the sensitivity and/or specificity of the test (23). Both LPA and LPI represent various subspecies with different fatty acid chains. In addition, the fatty acid chain may link to the glycerol backbone through different chemical linkages resulting in various subclasses [i.e., acyl (LPA), alkyl (A-LPA), and alkenyl (An-LPA)]. Findings of a study to evaluate the discriminating ability of LPA and LPI subspecies for ovarian cancer identification compared with total LPA and LPI suggested that subspecies with unsaturated fatty acid chains may be associated with late-stage or recurrent ovarian cancer (26). Other LPLs that have been proposed to have a biological role in ovarian cancer and be potentially useful as biomarkers of the disease include lysophosphatidylcholine (LPC), which has also been shown to be elevated in the plasma of ovarian cancer patients (27), and the lysosphingolipid sphingosine-1-phosphate (S1P), which is known to have both extracellular and intracellular signaling properties (28-31).

To further explore the potential of LPA, LPI, LPC, and S1P as biomarkers for ovarian cancer detection, we measured plasma LPL levels (including subspecies of LPA, LPI, and LPC) in women with ovarian cancer and healthy controls using an electrospray ionization mass spectrometry method recently developed by Xiao et al. (23). This assay allows simultaneous detection and quantitation of different species of LPL with at least 10 times more sensitivity than the previous gas chromatography method (23).

## Materials and Methods

**Patients.** All patient-derived biological specimens were collected under protocols approved by the University of South Florida Institutional Review Board, and all participants provided written informed consent.

Whole blood samples were obtained preoperatively in EDTA tubes by routine venipuncture of women undergoing surgery for suspected ovarian cancer in Hillsborough and Pinellas counties, Florida, between December 13, 2000 and October 30, 2002. All women ages 18 to 80 years undergoing surgery for suspected ovarian cancer in the two counties during the defined period were regarded as eligible for entry into the study. No patients who were asked refused to participate. Of the preoperative samples obtained, 45 were from women who were later confirmed to have ovarian cancer or primary peritoneal cancer (ovarian cancer patients; median age 60 years, range 33 to 79). Samples were obtained postoperatively from ovarian cancer patients from the same eligibility pool ( $n = 94$ , median age 59 years, range 26 to 80), including 22 patients who

had contributed a preoperative sample and 72 who had not. Whole blood samples from control subjects were collected concurrently from healthy women from the same counties who reported no history of cancer, gynecologic disease, oophorectomy or family history of breast/ovarian cancer ( $n = 27$ , median age 45 years, range 22 to 79). Whole blood specimens were obtained from a total of 117 ovarian cancer patients, including 18 patients with stage I disease, 11 with stage II disease, 74 with stage III disease, and 14 with stage IV disease. Among the 45 patients for whom a preoperative sample was available, there were 7 patients with stage I disease, 3 with stage II disease, 31 with stage III disease, and 4 with stage IV disease. Cancer diagnosis was confirmed for all cases by review of pathology records by a single ovarian cancer expert. Clinical stage was determined according to International Federation of Gynecologists and Obstetricians criteria (32), and the histologic subtype was evaluated according to the WHO classification (33).

**Sample Collection.** LPA is produced and released by activated platelets during coagulation and therefore is a normal constituent of serum, but it is present only at very low levels in whole blood or fresh platelet-poor plasma from healthy individuals (8). To prevent platelet activation and phospholipase activity, whole blood samples were collected via routine venipuncture in EDTA-containing tubes. Because LPLs are metabolites and levels may change during incubation, it is important that sample processing be as consistent as possible across all samples for comparison. We collected samples from multiple locations in the two study counties and processed (centrifugation and aliquoting) all samples at the University of South Florida H. Lee Moffitt Cancer Center and Research Institute (Moffitt). After blood drawing, samples were immediately chilled for transport to Moffitt by being placed in a Styrofoam container accompanied by a frozen pack for overnight delivery. This system allowed centrifugation within 16 to 28 hours after blood drawing. Samples appear stable for measurement of LPL when processed according to this protocol (Y. Xu, personal communication). Centrifugation was at  $3,000 \times g$  for 20 minutes after which the plasma was immediately aliquoted per each 0.5 mL into coated micro-Eppendorf tubes and immediately frozen at  $-70^{\circ}\text{C}$ . Samples were batch shipped on dry ice by overnight delivery to the Cleveland Clinic Foundation for analysis. Shipped samples were identified by a unique sample number only, without identifiers or any indication of the subject's status as ovarian cancer patient or control. The samples were maintained at  $-70^{\circ}\text{C}$  until preparation for mass spectrometry analysis. No personnel at the Cleveland Clinic Foundation had knowledge of the subjects' disease status at any time. Laboratory data were transmitted according to each unique sample number to Moffitt where all statistical analyses were performed.

**LPL Analysis.** Lipids were extracted as described previously with minor modifications (23, 34). To 0.5 mL plasma, 2 mL of MeOH/chloroform (2:1) and 0.1 mL of 6 N HCl were added. Samples were vortexed for 1 minute and incubated on ice for 10 minutes. Chloroform (1 mL) and  $\text{H}_2\text{O}$  (1 mL) were added to separate the phases. Samples were vortexed for 0.5 minute prior to centrifugation ( $2,000 \times g$  for 10 minutes). The lower phase was

transferred to a new glass tube. To the upper phase left in the original tube, 1 mL of chloroform was added to extract more lipids and the tube was centrifuged ( $2,000 \times g$  for 10 minutes). The lower phase was transferred into the same tube (with the lower phase extract), and the solvent was evaporated under nitrogen at  $30^\circ\text{C}$ . The dried lipids were suspended in 50  $\mu\text{L}$  of solvent (MeOH/chloroform 2:1), vortexed, and applied to a TLC plate. Two standards (18:1-LPA and 18:1-LPC) were applied to help in identifying the "LPA band" and "LPC band" on each TLC plate. The TLC plates were developed in the solvent system (chloroform/MeOH/amylic alcohol 65:35:5.5) until the solvent front was 1.5 inch from the top of the plate. The lipids from the "LPA band" and "LPC band" were eluted with 2 mL of MeOH/chloroform (2:1) twice. The lipid solutions were dried under nitrogen at  $30^\circ\text{C}$ , and lipids were resuspended in 100  $\mu\text{L}$  of MeOH for mass spectrometry.

Mass spectrometry analyses were performed using a Quattro Ultima triple quadrupole electrospray mass spectrometer (Micromass, Inc., Beverly, MA) with the MassLynx data acquisition system. A Waters 2690 (Waters, Milford, MA) autosampler was used to introduce the samples into the electrospray ionization source. The mobile phase used for all experiments was MeOH/ $\text{H}_2\text{O}$  (9:1 v/v), and the flow rate was 100  $\mu\text{L}/\text{min}$ . The injection volume was set to 20  $\mu\text{L}$  per sample for all experiments. The positive or negative ion mode with multiple reaction monitoring was used to quantitatively analyze the positively or negatively charged phospholipids. The collision energies were 70 eV in the negative mode and 25 eV in the positive mode. Nitrogen was used as both drying and nebulizing gas at flow rates of 500 and 50 L/h, respectively. The electrospray ionization probe capillary was held at 3 kV for the positive mode and \*3 kV for the negative mode, and the cone voltage was set at 35 V in positive mode and \*50 V in negative mode. The source and desolvation temperatures were  $100^\circ\text{C}$  and  $200^\circ\text{C}$ , respectively.

LPA and other negatively charged LPLs were analyzed in the negative mode with the monitoring ions at  $m/z$  378-79 (parent ion-product ion) for S1P, 381-79 for 14:0-LPA, 393-79 for 16:0-An-LPA, 395-79 for 16:0-A-LPA, 409-79 for 16:0-LPA, 421-79 for 18:0-An-LPA, 423-79 for 18:0-A-LPA, 433-79 for 18:2-LPA, 435-79 for 18:1-LPA, 437-79 for 18:0-LPA, 571-79 for 16:0-LPI, 599-79 for 18:0-LPI, and 619-79 for 20:4-LPI, respectively. All lipids with the phosphorylcholine group (positively charged) were analyzed in the positive mode. Monitoring ions were at  $m/z$  465-184 for SPC, 496-184 for 16:0-LPC, 510-184 for 17:0-LPC, 520-184 for 18:2-LPC, 524-184 for 18:0-LPC, 544-184 for 20:4-LPC and 568-184 for 22:6-LPC, respectively. The dwell time in the multiple reaction monitoring mode was 0.11 millisecond, and the scan delay was 0.02 second.

**Statistical Analysis.** Categorical variables were analyzed using  $\chi^2$  tests or Fisher's exact tests depending on sample size. Continuous variables, including univariate comparisons for quantitative variables between normal and cancer cases, were compared using the Student's  $t$  tests or the Wilcoxon rank sum test depending on the distribution of the variable of interest. Adjustment for potential confounding variables, such as the stage at diagnosis, was carried out by using general linear

modeling or ANOVA methods, as appropriate. Stepwise logistic regression analysis was used to determine the statistical significance of LPA, LPI, LPC (and their subspecies), and S1P. All statistical significance testing was two sided, and  $P < 0.05$  was considered to be statistically significant.  $P$  values in the 0.01 to 0.05 range should be interpreted with caution because of multiple testing issues. Statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC).

## Results

The ages, stages, grades, histologic subtypes, and treatment status of the 117 ovarian cancer patients who participated in the study are shown in Table 1. A total of 166 samples were analyzed including 27 from healthy controls, 45 obtained preoperatively from women with ovarian cancer, and 94 obtained postoperatively from women with ovarian cancer, with 22 patients having both preoperative and postoperative samples.

There were statistically significant differences between preoperative case samples ( $n = 45$ ) and control samples ( $n = 27$ ) in the mean levels of several individual LPA species, the combination of 16:0-LPA/20:4-LPA, total LPA, total LPI, and S1P (Table 2). The best discrimination between samples obtained preoperatively from ovarian cancer patients and those from healthy controls was achieved by the combined levels of 16:0-LPA and 20:4-LPA, with 93.1% correct classification, 91.1% sensitivity, and 96.3% specificity (Fig. 1). Receiver operating characteristic curves (35) were examined, and a cutoff 16:0-LPA/24:0-LPA level of 0.62  $\mu\text{mol}/\text{L}$  was identified as optimizing the sensitivity and specificity of the assay (Fig. 1). All patients with preoperative samples had

**Table 1. Clinical data for patients with ovarian cancer ( $n = 117$ )**

Characteristics	Stages I and II ( $n = 29$ )	Stages III and IV ( $n = 88$ )	Percentage ( $n = 117$ )
Age (y), median (range)	60 (32-77)	59 (26-80)	
Stages			
I	18	-	15.4
II	11	-	9.4
III	-	74	63.2
IV	-	14	12.0
Grades			
1	10	11	18.0
2	8	21	24.8
3	11	55	56.4
Ungraded	0	1	0.8
Histologic types			
Serous	12	61	62.4
Endometrioid	11	7	15.4
Mixed	0	8	6.8
Mucinous	3	2	4.3
Primary	0	4	3.4
peritoneal			
Clear cell	2	2	3.4
Transitional cell	1	2	2.6
Brenner	0	2	1.7
Treatment status			
Preoperative	10	35	38.5
Postoperative	19	53	61.5

Table 2. Mean (SD) for LPL in controls and preoperative case samples by stage ( $\mu\text{mol/L}$ )

Substance	Controls ( $n = 27$ )	Stage I ( $n = 7$ )	Stage II ( $n = 3$ )	Stage III ( $n = 31$ )	Stage IV ( $n = 4$ )
16:0-LPA*	00.14 (00.13)	00.52 (00.39)	00.62 (00.35)	00.73 (00.73)	00.37 (00.14)
18:0-LPA*	00.13 (00.10)	00.47 (00.42)	00.29 (00.19)	00.53 (00.51)	00.23 (00.03)
18:1-LPA*	00.17 (00.14)	00.37 (00.27)	00.46 (00.29)	00.47 (00.36)	00.32 (00.06)
18:2-LPA*	00.16 (00.14)	00.29 (00.26)	00.31 (00.08)	00.46 (00.39)	00.34 (00.09)
20:4-LPA*	00.22 (00.16)	00.71 (00.47)	00.31 (00.13)	00.50 (00.31)	00.55 (00.17)
22:6-LPA†	00.09 (00.07)	00.20 (00.12)	00.16 (00.09)	00.24 (00.24)	00.16 (00.03)
16:0-A-LPA‡	00.11 (00.08)	00.15 (00.07)	00.08 (00.05)	00.18 (00.08)	00.19 (00.04)
18:0-A-LPA§	00.04 (00.06)	00.07 (00.08)	00.10 (00.06)	00.08 (00.06)	00.07 (00.03)
16:0-An-LPA*	00.07 (00.05)	00.18 (00.11)	00.11 (00.01)	00.15 (00.10)	00.17 (00.05)
18:0-An-LPA*	00.03 (00.04)	00.07 (00.03)	00.11 (00.06)	00.09 (00.07)	00.04 (00.03)
Total A-LPA*	00.25 (00.12)	00.48 (00.13)	00.40 (00.10)	00.50 (00.19)	00.47 (00.04)
Total LPA*	00.90 (00.43)	02.57 (00.94)	02.15 (00.71)	02.93 (01.77)	01.97 (00.27)
16:0-LPA/20:4-LPA*	00.35 (00.17)	01.23 (00.52)	00.92 (00.43)	01.23 (00.70)	00.93 (00.15)
16:0-LPI†	00.49 (00.47)	00.75 (00.59)	01.88 (01.34)	01.00 (00.64)	00.90 (00.23)
18:0-LPI†	00.50 (00.43)	00.87 (00.71)	01.77 (02.49)	01.89 (02.05)	00.70 (00.25)
20:4-LPI*	00.51 (00.43)	01.35 (00.78)	00.93 (00.95)	01.36 (00.84)	01.36 (00.24)
Total LPI*	01.51 (00.79)	02.98 (01.57)	04.58 (02.71)	04.25 (02.81)	02.96 (00.33)
16:0-LPC	52.37 (25.63)	70.65 (30.07)	55.98 (26.57)	52.98 (30.62)	48.10 (21.15)
18:0-LPC	15.63 (08.28)	21.00 (09.90)	17.23 (10.98)	14.90 (09.56)	14.81 (06.57)
18:1-LPC	16.89 (07.27)	21.71 (10.42)	18.97 (13.40)	17.06 (11.40)	17.61 (10.02)
18:2-LPC‡	20.21 (07.63)	17.50 (07.72)	16.63 (12.86)	15.12 (08.99)	16.34 (10.36)
20:0-LPC	00.21 (00.07)	00.25 (00.12)	00.19 (00.08)	00.33 (00.41)	00.20 (00.14)
20:4-LPC	10.44 (03.10)	11.60 (04.95)	09.38 (01.56)	10.11 (04.72)	10.36 (03.41)
22:6-LPC‡	05.89 (02.24)	10.41 (06.00)	06.98 (04.63)	08.56 (05.96)	09.65 (05.96)
Total LPC	121.65 (47.22)	153.12 (60.02)	125.37 (68.84)	119.07 (64.40)	117.05 (57.06)
S1P†	00.36 (00.27)	00.77 (00.42)	00.50 (00.43)	00.66 (00.48)	00.65 (00.26)

NOTE: P values show significance levels for differences observed between healthy controls ( $n = 27$ ) and all ovarian cancer cases for whom preoperative samples were available ( $n = 45$ ).

\* $P < 0.0001$ .

† $P < 0.001$ .

‡ $P < 0.05$ .

§ $P < 0.01$ .

16:0-LPA/24:0-LPA levels above the 0.62  $\mu\text{mol/L}$  cutoff, with the exception of one stage I patient, one stage II patient, and two stage III patients. There were no significant differences in mean values for any LPL species between preoperative patients who were premenopausal versus postmenopausal. Levels did not correlate with tumor size. Using a receiver operating characteristic-derived cutoff value of 1.5  $\mu\text{mol/L}$ , total LPA levels achieved 91.7% correct classification, 91.1% sensitivity, and 92.6% specificity (Fig. 2). All four of the cases, which had 16:0-LPA/20:4-LPA levels below the 0.62  $\mu\text{mol/L}$  cutoff, also had low total LPA levels, as might be expected because total LPA includes 16:0-LPA and 20:4-LPA. Similarly, the control with an elevated 16:0-LPA/20:4-LPA level of 0.91  $\mu\text{mol/L}$  also had the highest total LPA level. CA 125 values were available on 35 of 45 patients with a preoperative sample. Levels were elevated  $>30$  units in 29 of 35 patients. Only one of six patients with a normal CA 125 preoperative value also had low (presumed normal) LPA values.

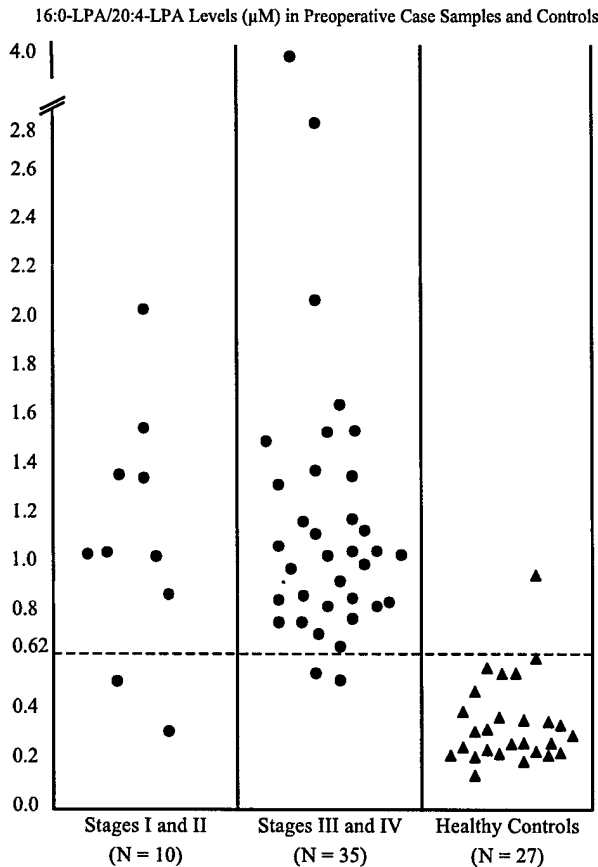
The mean (SD) values for the combination of 16:0-LPA/20:4-LPA in the plasma samples obtained preoperatively from patients with stages I to IV ovarian cancer were 1.23 (0.52), 0.92 (0.43), 1.23 (0.70), and 0.93 (0.15)  $\mu\text{mol/L}$ , respectively, compared with 0.35 (0.17)  $\mu\text{mol/L}$  for the controls (Table 2). The mean (SD) values of total LPA in the plasma samples obtained preoperatively from patients with stage I (7 patients), stage II (3 patients), stage III (31 patients), and stage IV (4 patients) ovarian cancer were 2.57 (0.94), 2.15 (0.71), 2.93 (1.77), and 1.97 (0.27)  $\mu\text{mol/L}$ , respectively, compared with 0.90 (0.43)  $\mu\text{mol/L}$  for 27 healthy controls (Table 2). The mean (SD)

values of total LPI in the plasma samples obtained preoperatively from patients with stages I to IV ovarian cancer were 2.98 (1.57), 4.58 (2.71), 4.25 (2.81), and 2.96 (0.33)  $\mu\text{mol/L}$ , respectively, compared with 1.51 (0.79)  $\mu\text{mol/L}$  for the controls (Table 2).

In 22 cases with both preoperative and postoperative samples, the postoperative levels of total LPA, total LPC, 22:6-LPA, 18:0-LPA, the combination of 20:4-LPA/22:6-LPA, 20:4-LPC, and 18:2-LPC were significantly lower than preoperative levels ( $P = 0.03, 0.05, 0.02, 0.04, 0.03, 0.02, 0.003, \text{ and } 0.03$ , respectively; Table 3). Of these LPLs, 18:0-LPC, 18:2-LPC, and total LPC levels also showed statistically significant differences between preoperative case samples ( $n = 45$ ) and all postoperative case samples ( $n = 94$ ;  $P \leq 0.05$ ). There were no statistically significant differences in mean LPL levels between postoperative samples obtained prior to initiation of chemotherapy versus postchemotherapy.

## Discussion

Ovarian cancer is a disease associated with a high mortality mainly because it currently escapes detection at early stages. Identification of an effective biomarker for early detection would improve survival. This study reports statistically significant differences in LPL levels between preoperative samples of ovarian cancer patients and those of healthy controls. The study also confirms that statistically significant elevations in LPL levels are present in patients with early-stage disease. Thus, the findings support the utility of LPL, especially LPA, as



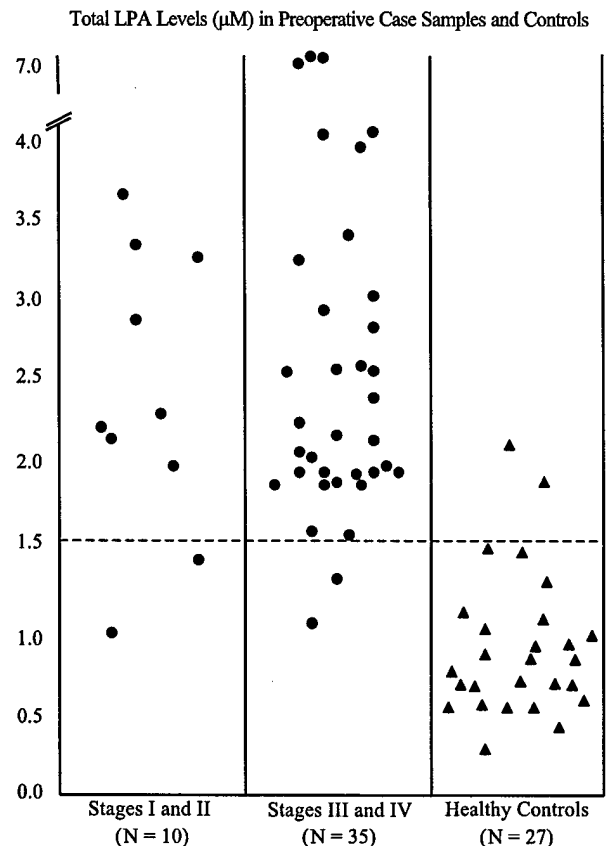
**Figure 1.** 16:0-LPA/20:4-LPA levels ( $\mu\text{mol/L}$ ) in preoperative case samples and controls.

biomarkers for early detection of ovarian cancer. The study is the first to report significant postoperative changes in specific LPL levels. Further study is needed to determine whether some LPLs may return to baseline after successful treatment and/or have utility as biomarkers of recurrence. The study also contributes data toward determination of the best combinations of markers and cutoff values for clinical use.

Although our conclusions are still preliminary because our study sample is small and not ideal for demonstrating the value of LPL for screening, our findings regarding the utility of LPL as biomarkers of ovarian cancer are critically important because the two previous studies showed conflicting results (8, 10). To ensure the validity of our data, only investigators at Moffitt had access to clinical data, and the investigators performing LPL measurements at Cleveland Clinic Foundation were blinded to the case versus control status of the samples. All statistical analyses were performed at Moffitt.

The reason for the discrepancy between the findings of the two prior studies with interpretable results regarding the utility of LPA as a biomarker for detection of ovarian cancer is unclear. There were many methodological differences between the two studies, including differences in sample collection, processing, and lipid analyses (8, 10). Our experience suggests that it is critical to maintain consistency of procedures for all samples to be

compared, including the time and temperature prior to and during centrifugation, sample storage vials (see below), extraction solvents and methods, establishment of standard curves, and mass spectroscopy methods. The following example demonstrates the importance of these aspects. Prior to analyzing the samples included in this report, we analyzed a batch of samples ( $n = 33$ ) that showed lower overall LPL levels than anticipated among both cases and controls, with less separation than anticipated between levels of cases and those of controls. These findings prompted a review of procedures. Our review identified that the type of micro-Eppendorf tubes used for storage after centrifugation was critically important. If the tubes were not siliconized or pre-lubricated, as much as 90% of negatively charged LPLs were absorbed into the tube walls. Further analysis was performed, including paired storage of identical samples using coated and uncoated tubes, with the resulting differences in LPL levels analyzed. The analysis confirmed that the difference in tubes accounted for the differences in levels observed; therefore, data from these samples were not included in the analyses (data not shown). The following suggestions are offered for future investigations of LPL: we recommend use of SafeSeal microcentrifuge tubes (catalogue 505-201, PGC Scientifics, Frederick, MD) for plasma storage and use of glassware only (not plastic ware), except for the storage tubes mentioned above.



**Figure 2.** Total LPA levels ( $\mu\text{mol/L}$ ) in preoperative case samples and controls.

**Table 3. Mean (SD) for paired preoperative and postoperative samples (n = 22)**

Substance	Preoperative Mean	Postoperative Mean
16:0-LPA	00.85 (00.84)	00.50 (00.28)
18:0-LPA*	00.64 (00.61)	00.33 (00.24)
18:1-LPA	00.55 (00.41)	00.36 (00.29)
18:2-LPA	00.39 (00.43)	00.38 (00.27)
20:4-LPA	00.55 (00.39)	00.47 (00.41)
22:6-LPA*	00.28 (00.28)	00.12 (00.09)
16:0-A-LPA	00.17 (00.09)	00.16 (00.15)
18:0-A-LPA	00.10 (00.06)	00.09 (00.10)
16:0-An-LPA	00.14 (00.07)	00.14 (00.11)
18:0-An-LPA	00.09 (00.07)	00.06 (00.07)
Total A-LPA	00.50 (00.18)	00.44 (00.27)
Total LPA*	03.27 (01.98)	02.16 (01.04)
16:0-LPA/20:4-LPA*	01.41 (00.78)	00.97 (00.51)
16:0-LPI	01.21 (00.91)	01.24 (01.40)
18:0-LPI	02.06 (02.32)	01.28 (01.37)
20:4-LPI	01.38 (00.99)	01.34 (01.06)
Total LPI	04.65 (03.21)	03.86 (02.05)
16:0-LPC	52.61 (30.34)	67.32 (36.06)
18:0-LPC	13.72 (08.62)	18.96 (10.18)
18:1-LPC	15.08 (09.13)	20.95 (10.90)
18:2-LPC†	13.95 (08.49)	21.67 (07.76)
20:0-LPC*	00.30 (00.43)	00.38 (00.64)
20:4-LPC	09.51 (04.68)	13.19 (05.32)
22:6-LPC	07.64 (05.69)	09.13 (04.77)
Total LPC*	112.81 (59.37)	151.60 (67.52)
SIP*	00.78 (00.54)	00.48 (00.29)

NOTE: are indicated.

\*P &lt; 0.05, statistically significant differences between preoperative mean values and postoperative mean values.

† P &lt; 0.01, statistically significant differences between preoperative mean values and postoperative mean values.

Further studies are under way to evaluate specificity of LPL measurements obtained not only from healthy controls but also from women with benign gynecologic disease, other gynecologic cancers, and nongynecologic cancers. Additional studies are planned to evaluate LPL measurements in combination with other markers, including proteomic markers (7) and algorithms of changes in CA 125 values over time (36). Longitudinal data will allow us to evaluate whether and when specific LPL return to baseline after successful treatment and their utility in predicting recurrence. Studies are also needed to specifically address the utility of LPL measurements in women at hereditary risk for ovarian cancer, a group in whom early detection is desperately needed but in whom baseline LPL levels may differ from healthy women at average risk (unpublished preliminary data). Thus, larger studies with the capability of yielding more precise estimates of the sensitivity and specificity of LPL, both alone and in combination with other markers, for both screening and detection of recurrence are necessary.

In summary, our findings support the potential of LPL levels as biomarkers of ovarian cancer, specifically LPA levels as diagnostic markers. However, these findings require validation in larger studies.

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### References

- American Cancer Society. Cancer facts and figures 2003. American Cancer Society [accessed 2003 Apr]. Available from: [http://www.cancer.org/docroot/STT/stt\\_0.asp](http://www.cancer.org/docroot/STT/stt_0.asp).
- Mok SC, Chao J, Skates S, et al. Prostate, a potential serum marker for ovarian cancer: identification through microarray technology. *J Natl Cancer Inst* 2001;93:1458-64.
- Schwartz PE, Taylor KJ. Is early detection of ovarian cancer possible? *Ann Med* 1995;27:519-28.
- Taylor KJ, Schwartz PE. Screening for early ovarian cancer. *Radiology* 1994;192:1-10.
- Greenlee RT, Hill-Harmon MB, Murray T, Thun M. Cancer statistics, 2001. *CA Cancer J Clin* 2001;51:15-36.
- Bast RC Jr, Xu FJ, Yu YH, Barnhill S, Zhang Z, Mills GB. CA 125: the past and the future. *Int J Biol Markers* 1998;13:179-87.
- Petricoin EF, Ardekani AM, Hitt BA, et al. Use of proteomic patterns in serum to identify ovarian cancer. *Lancet* 2002;359:572-7.
- Xu Y, Shen Z, Wiper DW, et al. Lysophosphatidic acid as a potential biomarker for ovarian and other gynecologic cancers. *JAMA* 1998;280:719-23.
- Kim H, Hye-Ran Y, Pyo D. Quantitative analysis of lysophosphatidic acid in human plasma by tandem mass spectrometry. *Bull Korean Chem Soc* 2002;23:1139-43.
- Baker DL, Morrison P, Miller B, et al. Plasma lysophosphatidic acid concentration and ovarian cancer. *JAMA* 2002;287:3081-2.
- Xu Y, Fang XJ, Casey G, Mills GB. Lysophospholipids activate ovarian and breast cancer cells. *Biochem J* 1995;309 Pt 3:933-40.
- Xu Y, Gaudette DC, Boynton JD, et al. Characterization of an ovarian cancer activating factor in ascites from ovarian cancer patients. *Clin Cancer Res* 1995;1:1223-32.
- Gaits F, Salles JP, Chap H. Dual effect of lysophosphatidic acid on proliferation of glomerular mesangial cells. *Kidney Int* 1997;51:1022-7.
- Gennaro I, Xuereb JM, Simon MF, et al. Effects of lysophosphatidic acid on proliferation and cytosolic Ca<sup>2+</sup> of human adult vascular smooth muscle cells in culture. *Thromb Res* 1994;94:317-26.
- Goetzl EJ, Dolezalova H, Kong Y, Zeng L. Dual mechanisms for lysophospholipid induction of proliferation of human breast carcinoma cells. *Cancer Res* 1999;59:4732-7.
- Imamura F, Mukai M, Ayaki M, et al. Involvement of small GTPases Rho and Rac in the invasion of rat ascites hepatoma cells. *Clin Exp Metastasis* 1999;17:141-8.
- Genda T, Sakamoto M, Ichida T, et al. Cell motility mediated by rho and Rho-associated protein kinase plays a critical role in intrahepatic metastasis of human hepatocellular carcinoma. *Hepatology* 1999;30:1027-36.
- Manning TJ Jr, Parker JC, Sontheimer H. Role of lysophosphatidic acid and rho in glioma cell motility. *Cell Motil Cytoskeleton* 2000;45:185-199.
- Mukai M, Imamura F, Ayaki M, et al. Inhibition of tumor invasion and metastasis by a novel lysophosphatidic acid (cyclic LPA). *Int J Cancer* 1999;81:918-22.
- Panetti TS, Chen H, Misenheimer TM, Getzler SB, Mosher DF. Endothelial cell mitogenesis induced by LPA: inhibition by thrombospondin-1 and thrombospondin-2. *J Lab Clin Med* 1997;129:208-16.
- Ren XD, Kiosses WB, Schwartz MA. Regulation of the small GTP-binding protein Rho by cell adhesion and the cytoskeleton. *EMBO J* 1999;18:578-85.
- Sengupta S, Xiao Y, Xu Y. A novel laminin-induced LPA autocrine loop in the migration of ovarian cancer cells. *FASEB J* 2003;17:1570-2.
- Xiao Y, Chen Y, Kennedy AW, Belinson J, Xu Y. Evaluation of plasma lysophospholipids for diagnostic significance using electrospray ionization mass spectrometry (ESI-MS) analyses. *Ann NY Acad Sci* 2000;905:242-59.
- Liscovitch M, Cantley LC. Lipid second messengers. *Cell* 1994;77:329-34.
- Moolenaar WH, Kranenburg O, Postma FR, Zondag GC. Lysophosphatidic acid: G-protein signaling and cellular responses. *Curr Opin Cell Biol* 1997;9:168-73.
- Shen Z, Wu M, Elson P, et al. Fatty acid composition of lysophosphatidic acid and lysophosphatidylinositol in plasma from patients with ovarian cancer and other gynecological diseases. *Gynecol Oncol* 2001;83:25-30.
- Okita M, Gaudette DC, Mills GB, Holub BJ. Elevated levels and altered fatty acid composition of plasma lysophosphatidylcholine (lysoPC) in ovarian cancer patients. *Int J Cancer* 1997;71:31-4.
- Van Brocklyn JR, Lee MJ, Menzelev R, et al. Dual actions of sphingosine-1-phosphate: extracellular through the Gi-coupled receptor Edg-1 and intracellular to regulate proliferation and survival. *J Cell Biol* 1998;142:229-40.

29. Spiegel S, Milstien S. Sphingolipid metabolites: members of a new class of lipid second messengers. *J Membr Biol* 1995;146:225-37.
30. Meyer zu Heringdorf D, van Koppen CJ, Jakobs KH. Molecular diversity of sphingolipid signaling. *FEBS Lett* 1997;410:34-8.
31. Spiegel S. Sphingosine 1-phosphate: a prototype of a new class of second messengers. *J Leukoc Biol* 1999;65:341-4.
32. Ozols RF, Rubin SC, Thomas G, Robboy S. Epithelial ovarian cancer. In: Hoskins WJ, Perez CA, Young RC, editors. *Principles and principles of gynecologic oncology*. Philadelphia: Lippincott-Raven Publishers; 1997. p. 958.
33. WHO. WHO Handbook for reporting results of cancer treatment. Geneva (Switzerland): WHO; 1979.
34. Xiao YJ, Schwartz B, Washington M, et al. Electrospray ionization mass spectrometry analysis of lysophospholipids in human ascitic fluids: comparison of the lysophospholipid contents in malignant vs nonmalignant ascitic fluids. *Anal Biochem* 2001;290:302-13.
35. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem* 1993;39:561-77.
36. Jacobs IJ, Skates SJ, MacDonald N, et al. Screening for ovarian cancer: a pilot randomized controlled trial. *Lancet* 1999;353:1207-10.

**Lung Cancer Screening with Computed Tomography:  
Initial Results of a Cohort Screening Trial**

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**Lung Cancer Screening with Computed Tomography:  
Initial Results of a Cohort Screening Trial**

**Abstract**

**Purpose:** This paper reports our initial results from an ongoing, prospective, longitudinal, single-arm, clinical trial of lung cancer screening with computed tomography (CT).

**Materials and Methods:** Eligible subjects were asymptomatic women and men 45 years of age or older, with a history of cigarette smoking of at least 30 pack-years. The screening study design includes one baseline screening round and four subsequent annual screening rounds.

**Results:** The cohort consists of 1151 enrolled subjects. Overall, 59% of subjects were male and 41% female, and the mean age was 60.2 years. The mean pack-years of smoking history were 57.9 pack-years. Baseline screening detected 28 neoplasms, and 25 cases of non-small cell lung cancer (NSCLC) (2.2% prevalence rate). Of baseline NSCLC cases, 14 were stage 1 (56%), 12 stage 1A. To date in the study, 18 incidence cancers have been detected, 15 of which are NSCLC; 10 of these cases were stage 1 (66%). Overall, 60% of detected lung cancers were stage 1.

**Conclusion:** Our results confirm that screening CT identifies small and early-stage lung cancers. However, small cancer size does not always correlate with early stage disease.

**Key Words:** screening; computed tomography; lung cancer; outcomes analysis

## Introduction

Lung cancer is the leading cause of cancer deaths for both men and women in the United States. In 2004, about 160,000 men and women will die from lung cancer, more deaths than from breast, prostate, cervix and colon cancers combined <sup>1</sup>.

In spite of advances in treatment, the survival rate for lung cancer has not changed in the past 30 years. In the United States, the overall five-year survival rate remains a dismal 14% and the ten-year survival rate is 7% <sup>2</sup>. However, survival is related to stage at presentation. The five-year survival rate for patients with localized (node negative) tumors is over 50% <sup>3,4</sup> while the survival rate for stage 1A (T1N0M0) disease is 60-80% <sup>5,6,7</sup>.

Unfortunately, very few lung cancers in the United States are detected early. More than 50% of patients will have distant metastases at diagnosis and only 25% will be localized and potentially resectable for cure <sup>8</sup>. Effective methods of early detection, therefore, might improve lung cancer survival and mortality rates.

Previous randomized, controlled trials of lung cancer screening with chest radiography alone or in combination with sputum cytology demonstrated no mortality reduction benefit to screened groups <sup>9,10,11,12,13,14</sup>, even though screened groups had more and smaller lung cancers detected, more surgically resectable cancers, and greater 5-year survival rates than control groups. These effects have been ascribed to a combination of lead-time, length, and overdiagnosis biases. Therefore, currently, no organizations recommend screening for lung cancer.

However, interest in lung cancer screening has been revived because of recent reports from several clinical trials using low-dose, single breath, helical computed tomography (CT) <sup>15,16,17,18,19,20,21</sup>. These studies have shown that screening with CT can

detect lung cancers at smaller size, and earlier stage, than chest radiography and current clinical practice.

Several years ago we initiated a prospective, longitudinal, single-arm cohort screening trial, with the hypothesis that screening with CT and sputum molecular markers will increase the proportion of stage 1 cancers by at least three-fold compared to current clinical practice and to over 60% of total cohort lung cancers. The study design includes one baseline (prevalence) screen, and four subsequent annual repeat (incidence) screening rounds. The trial is now partially complete with over 50% of the screening events completed. The purpose of this paper is to report our results to date of screening for lung cancer with CT.

### **Material and Methods**

Our university Institutional Review Board (IRB) and the IRB of the Department of Defense (DOD), the research grant funding agency, approved the study protocol. Participants were enrolled into the study after meeting eligibility requirements, and after giving written informed consent.

The number of subjects to be recruited (1,150) was calculated to enable detection of at least 50 lung cancers over the course of the trial, assuming a lung cancer detection rate of 1.2%. Participants for the study were self-selected, and recruited through promotions at local television news stations, health fairs, newspaper advertisements, and by referral from personal physicians.

Eligible subjects were asymptomatic women and men 45 years of age or older. Participants had to be current or former cigarette smokers, with a history of cigarette smoking of at least 30 pack-years. Ineligible were those with a history of any cancer other than non-melanoma skin cancer. Only mentally competent patients considered healthy enough to

undergo pulmonary resection (i.e., patients without congestive heart failure or disabling dyspnea at the time of enrollment) were entered into the study.

All participants agreed to undergo spirometry, with measurement of forced expiratory volume in 1 second (FEV<sub>1</sub>) and forced vital capacity (FVC), a venous blood specimen, induced sputum collection plus a 3-day mailer for spontaneously produced sputum, and a CT scan at each annual visit. Since cigarette smokers with impaired expiratory airflow (pulmonary obstruction) have a 4-6 fold excess risk for lung cancer compared to non-obstructed cigarette smokers<sup>22</sup>, the initial eligibility requirements included a degree of pulmonary obstruction (FEV<sub>1</sub> ÷ FVC ≤ 70%, termed "obstructed" subjects). Enrollment was interrupted for 9 months due to transfer of research grant administration from the Navy to the Army within the DOD. This transfer required re-review and approval by another DOD IRB and delayed subsequent completion of enrollment of the entire cohort. Further, after preliminary review the prevalence of lung cancer exceeded the design requirement. To facilitate accrual, the spirometry eligibility requirement was revised, and all spirometry values were permitted, including FEV<sub>1</sub> ÷ FVC > 70% (termed "unobstructed" subjects).

Screening CT examinations were performed using single channel helical (Siemens Somaris software) and multichannel helical (Siemens Syngo software) scanners. Subjects were scanned with a table movement of 20 mm/sec, a 2:1 pitch, 120 kVp, mA ranging from 20 - 80, and reconstructed image thickness of 10mm. Screening scans were done with a single breath-hold acquisition without intravenous contrast medium.

Diagnostic follow-up CT examinations were performed using the same single channel and multichannel helical scanners. Subjects were scanned during a single breath-hold with a table movement of 20 mm/sec, a 2:1 pitch, 120-140 kVp and mA ranging from 80-240.

Diagnostic CT examinations for suspicious lesions felt to have a high likelihood of being lung

cancer were done with intravenous contrast administration with images reconstructed at contiguous 8mm intervals. For follow-up of probably benign nodules, non-contrast low-dose scans through the lungs were used to localize the nodules for thin-section CT (1-2 mm).

All CT images were interpreted at a PACS (picture/archive/communication system workstation) (Siemens MagicView 1000) independently by one of three investigator radiologists. Images were viewed in both lung (W 2000, L -600) and soft tissue (W 350, L 20) window/level settings. The location and size of any non-calcified nodule or opacity, and any additional findings were reported.

The criteria for an abnormal screening CT examination included any noncalcified pulmonary or hilar mass, nodule or opacity of any size that could represent lung cancer. Obvious scars, isolated small pleural effusions, emphysema, and other non-neoplastic findings were reported but not tabulated as an abnormal screening CT. Vascular or extrathoracic findings were reported and recommendations made for clinical evaluation, but these also were not tabulated as an abnormal screening CT for lung cancer.

Written diagnostic management recommendations for CT findings were made in each case to the attending physician based on a management algorithm for indeterminate lung nodules or opacities, similar to those used in other cohort CT screening trials (Figure 1). In addition, each case of a nodule or opacity greater than 8 mm in greatest diameter was reviewed at a multi-disciplinary conference of radiologists, pulmonologists, thoracic surgeons, and radiation oncologists to achieve consensus in subsequent management recommendations. However, subsequent diagnostic and treatment decisions were at the direction of the primary physician and the patient.

## Results

From December 4, 1998 to October 10, 2002, 1,151 participants were enrolled and underwent the baseline prevalence CT scan. Enrollment was denied to 2,345 applicants because they did not meet the eligibility criteria. The reasons for ineligibility included age, insufficient smoking history, history of cancer, not interested in participating, lack of transportation, and miscellaneous health or personal issues.

The characteristics of the subjects enrolled in our trial are summarized in Table 1. Overall, 59% of subjects were male and 41% female, and the mean age was 60.2 years. The mean pack-years of smoking history were 57.9 pack-years. Subjects with pulmonary obstruction were more often male, were older, and had greater pack-years smoking history, than those subjects without obstruction.

### Baseline Screening Round

In our screened cohort, 406 (35%) of the subjects had an abnormal baseline prevalence CT, and all received subsequent diagnostic evaluation (Table 2).

Baseline screening CT detected 28 neoplasms (2.4% of 1151 subjects), including one non-Hodgkin lymphoma and two small cell lung carcinoma (SCLC). Surgery yielded a benign diagnosis in 3 patients: 2 benign non-caseating granulomas, 1 aspergillus granuloma.

There were 25 cases of non-small cell lung cancer (NSCLC) detected in the prevalence screen (prevalence NSCLC rate 2.2% of 1151 subjects), and all had complete staging and pathological review. Surgical pulmonary resection and mediastinal lymphadenectomy of NSCLC was performed in 20 participants, while neoplasm was diagnosed and staged without surgery in 8 patients (5 with stage 4 NSCLC, 1 lymphoma, and 2 SCLC).

The characteristics of the patients with NSCLC detected in the baseline screening are summarized in Table 3. Twenty-two of the 25 (88%) NSCLC cases occurred in subjects with pulmonary obstruction. There was a 5-fold difference in prevalence cancer rates between obstructed and unobstructed subjects. The prevalence cancer rate in obstructed subjects was 3.2%; in unobstructed subjects, 0.64%. Even though 59% of the cohort subjects were male, NSCLC was detected in the baseline screening round more often in women (52% of NSCLC) than in men ( $p = 0.24$ ).

The size, stage, and cell type distributions of the cancers detected in the baseline screening round are summarized in Table 4. There were 14 stage 1 cancers (56%) with 12 stage 1A.

The mean tumor size (T size) of NSCLC detected in the baseline round was 21 mm (9-60 mm). Nineteen of the 25 cases (76%) of NSCLC were T1 cancers ( $\leq 30$  mm.); the mean size of T1 cancers was 16 mm (9-30 mm). Advanced stage cancers (stages 3 and 4) were also relatively small (mean size 19 mm), and 5 of 7 such cancers were  $\leq 20$  mm. in diameter.

#### Incidence Screening Rounds

In the first complete incidence screening round, 930 subjects returned for their annual screening CT (83% participation); 130 (14%) of the subjects had an abnormal screening CT, and all received subsequent diagnostic evaluation.

The first incidence screening round detected 8 neoplasms (incidence rate 0.7 % of 1151 subjects), including one SCLC; all had complete staging and pathological review. There were 4 stage 1 NSCLC cases.

Although the second through fourth incidence screening rounds are not yet complete, we have completed 57% of expected screenings (Table 2). The incidence screenings have to date detected 18 neoplasms, including three SCLC. Surgery yielded a benign diagnosis

in 9 patients with new and enlarging nodules detected on incidence screening: 5 benign non-caseating granulomas, 1 aspergillus granuloma, 2 necrotizing granulomas, and 1 intrapulmonary lymph node.

The characteristics of the patients with incidence screening detected cancers is summarized in Table 2. All incidence cancers were detected in subjects with pulmonary obstruction. The size, stage, and cell type distributions of the cancers detected in the incidence screening rounds are summarized in Table 4. Sixty-six percent of the incidence NSCLC were stage 1.

The mean tumor size (T size) of NSCLC detected in the incidence screening rounds was 22 mm (8-35 mm). Twelve of the 15 cases (75%) of incidence NSCLC were T1 cancers.

PET scans were performed in 31 of 46 patients with screening-detected neoplasms. The mean tumor diameter of NSCLC cases with PET imaging was 24 mm; 22 of 31 cases (71%) were T1 cancers, and 11 (35%) were less than 15 mm. in diameter. For the PET diagnosis of neoplasm, there were 26 true positives (Figure 2), 1 false positive (Figure 3), 6 true negatives, and 5 false negative studies (Figure 4): sensitivity = 84%, specificity = 86%, positive predictive value = 96%, and negative predictive value = 55%. In 4 of the 5 false negative cancers, the CT appearance of the pulmonary nodule was a ground-glass opacity rather than a solid nodule (Figure 4). In each of these cases the cancer was bronchoalveolar cell type.

## **Discussion**

The results of our prospective cohort trial indicate that CT can identify small and early-stage lung cancers. Most of the non-small lung cancers detected by computed tomography were stage I at diagnosis. This currently fulfills our stated hypothesis that CT screening would demonstrate a 3-fold increase in the proportion of stage 1 cancers detected by

screening compared to current clinical practice and to over 60% of total cohort lung cancers. We must await further incidence screening results to determine if this rate of early stage diagnosis is maintained and accompanied by a lower frequency of advanced stage cancers (i.e., a stage shift). At least one economic model<sup>23</sup> suggests that if detection of 60% of lung cancer in stage I were accompanied by a reduction in the lung cancer mortality rate, that screening would be cost-effective.

False positive screening CT examinations are a considerable concern. In our prevalence round of screening, the positive predictive value for interpretation ( $PPV_1$ )<sup>23</sup> of an abnormal screening CT for neoplasm was 6.9% ( $PPV_1 = \text{true positive cases} \div \text{abnormal screening exams} = 28/406$ ). In the 1<sup>st</sup> incidence screening round,  $PPV_1 = 6.2\%$  (8/130). This is similar to the expected  $PPV_1$  for breast cancer screening: 5-10%<sup>24,25</sup>, an accepted and widespread screening practice. Although our "recall rate"<sup>23,24</sup> for CT screening (35% prevalence, 14% incidence) is higher than that expected for mammography screening (<10%<sup>24</sup>), the lung cancer yield is greater than the breast cancer yield, so the predictive values are similar. Our positive predictive value for biopsy in the prevalence screening round ( $PPV_2$ ) was 90% ( $PPV_2 = \text{cases of neoplasm at biopsy} \div \text{all biopsy cases} = 28/31$ ) and in the first incidence round was 73%, both higher than that expected for breast cancer screening: 20-40%<sup>23,24</sup>.

Because of the high rate of false positive screenings, non-invasive diagnostic strategies, such as PET imaging are appealing. However, although there is extensive scientific data supporting the use of PET for lung cancer staging<sup>26</sup>, recommendations for its use in evaluation of the solitary pulmonary nodule are mixed<sup>27,28,29,30,31</sup>. The limitations of PET for this indication include a) very little data about imaging nodules less than 10mm in

diameter, b) risk of false positive studies in inflammatory nodules with high metabolic activity, c) risk of false negative studies in neoplasms with low metabolic activity, and d) accuracy dependent upon a *priori* probability of the nodule being neoplasm. All of these potential limitations would apply to screening-detected pulmonary nodules.

One previous report directly addresses the accuracy of PET imaging in the diagnosis of selected screening-detected pulmonary nodules<sup>21</sup>. Pastorino, et. al. reported their results with PET for the diagnosis of 42 screening-detected lesions, with a sensitivity of 90%, specificity 82%, positive predictive value 82%, and negative predictive value 90%. Our experience is somewhat different, with a lower negative predictive value (55%) because of our false negative examinations. Our analysis of the false negative studies reveals that 80% occurred in lesions that appeared as ground-glass opacities on CT, rather than solid nodules. Therefore, PET imaging in the diagnosis of screening-detected pulmonary nodules is useful, if its limitation in evaluation of ground-glass opacities is recognized, and its utility applied to solid nodules.

Unfortunately, small cancer size does not always mean early stage. In our series, 78% (31/40) of screening detected NSCLC were T1 lesions, yet only 60% (24/40) were stage 1. Therefore, 23% (7/31) of our screening-detected T1 NSCLC were stages 2 - 4 at diagnosis. This is similar to other published reports of the stage distribution of small lung cancers, in which metastases were present in 17%-40% of T1 lung cancers<sup>32, 33, 34</sup> and 18% of cancers  $\leq 10$  mm.<sup>35</sup> Moreover, when T1 cancers are stratified by size, there is no correlation between tumor size and survival<sup>36</sup>. Size of tumor alone therefore may not be an adequate measure of either biologic activity, or probability of regional and distant metastatic spread.

The major hypothesis of CT screening is that cancer stage distribution depends primarily on tumor size at detection; i.e., detecting smaller tumors will result in a greater proportion of Stage I disease. However, our results and the results of others suggest that small tumor size is only one component of early stage, casting some doubt that this crucial hypothesis is complete.

Consideration of metastatic potential therefore may be as important as lesion size for successful screening. We are concurrently collecting sputum annually from our cohort subjects to test complementary biomarkers of metastatic potential during this trial of helical CT screening. Ongoing trials of lung cancer screening which combine both helical CT and biomarker collection offer additional opportunities to consider metastatic potential of detected cancers. Such a multi-center trial has begun in the United States: the National Lung Screening Trial (NLST) funded by the National Cancer Institute (NCI)<sup>37, 38</sup>, a randomized, controlled trial that compares screening with CT to screening with chest radiography. The enrollment criteria are women and men smokers or former smokers, ages 55 – 74 years and  $\geq 30$  pack-years of smoking history. The NLST measurement endpoints are mortality, quality of life and cost-effectiveness. Specimens of blood, sputum, and urine collected during the NLST may determine whether evaluation of lung cancer biological behavior contributes to successful screening.

The enrollment eligibility criteria of the reported trials vary greatly. For example, the *mean* age of subjects enrolled in our study (60.2 years) is the *minimum* age of enrollment in the ELCAP study<sup>15</sup> (60 years). Selection bias probably exists in these single-arm cohort studies, including our own, since the potential for bias exists in all single-arm studies. The relatively high percentage of stage IA NSCLC detected with CT could reflect any combination of selection, length, overdiagnosis, and lead-time biases<sup>39, 40, 41, 42, 43, 44, 45, 46</sup>. To demonstrate

a stage shift with CT screening, one must show not only an increase in early-stage disease but also a concomitant decrease in late-stage disease. Further incidence screening data and more years of clinical follow-up are necessary to determine if this occurs.

**TABLE 1: Characteristics of subjects enrolled in the screening cohort**

	<b>Total Cohort</b>	<b>Obstructed *</b> <b>Subjects</b>	<b>Unobstructed **</b> <b>Subjects</b>
<b>Age (years) +/- SD</b>	60.2 +/- 8.4	62.0 +/- 8.3	57.6 +/- 8.0
<b>Sex (male/female)</b>	684 / 467	414 / 268	270 / 199
<b>Mean pack-years smoking history +/- SD</b>	57.9 +/- 26.0	62.1 +/- 27.3	51.6 +/- 22.5
<b>Mean FEV<sub>1</sub>/FVC +/- SD</b>	65.5 +/- 13.0	57.5 +/- 10.7	77.3 +/- 4.4
<p><b>* Obstructed: FEV<sub>1</sub> / FVC ≤ 70%</b>  <b>** Unobstructed: FEV<sub>1</sub> / FVC &gt; 70%</b></p> <p><b>SD = standard deviation</b>  <b>FVC = forced vital capacity</b>  <b>FEV<sub>1</sub> = forced expiratory volume in 1 second</b></p>			

**TABLE 2: Characteristics of patients with screening-detected NCLC**

	Prevalence Cancers (n = 25)		Incidence Cancers (n = 15)	
	Obstructed *	Non-Obstructed **	Obstructed *	Non-Obstructed **
<b>NSCLC Cases (number)</b>	22	3	15	0
<b>Age (years (+/- SD))</b>	64.5 +/- 8.2	59 +/- 12.7	65.8 +/- 7.1	*
<b>Sex (male/female)</b>	12/10	0/3	11/4	*
<b>Mean pack-years smoking history (+/- SD)</b>	62.2 +/- 25.2	47.8 +/- 6.7	76.6 +/- 27.3	*
<b>Mean FEV1/FVC (+/- SD)</b>	59.5 +/- 10.7	71.6 +/- 0.3	53.4 +/- 11.1	*
<p>* Obstructed: FEV1 / FVC <math>\leq</math> 70%</p> <p>** Unobstructed: FEV1 / FVC &gt; 70%</p> <p>NSCLC = non-small cell lung cancer</p> <p>SD = standard deviation</p> <p>FVC = forced vital capacity</p> <p>FEV1 = forced expiratory volume in 1 second</p>				

**TABLE 3: Summary Results of On-going Screening Trial**

	<b>Screening Round</b>					<b>Screening Events</b>
	<b>Prevalance</b>	<b>Incidence #1</b>	<b>Incidence #2</b>	<b>Incidence #3</b>	<b>Incidence #4</b>	
<b>Expected Screenings</b>	1151	1123	1115	1108	1106	5603
<b>Completed screenings</b>	1151	930	612	356	140	3189
<b>% Completion</b>	100%	83%	55%	32%	13%	57%
						<b>Total</b>
<b># Abnormal Screening</b>	406	130	76	50	18	680
<b>Abnormal Screening Rate</b>	35%	14%	12%	14%	13%	21%
<b># Neoplasms detected</b>	28	8	7	2	1	46
<b>Neoplasm Detection Rate</b>	2.4%	0.7%	0.6%	0.2%	0.1%	0.8%
<b>PPV1 (interpretation)</b>	6.9%	6.2%	9.2%	4.0%	5.6%	6.8%
<b># Benign Biopsies</b>	3	3	2	4	0	12
<b>% Benign Biopsies</b>	10%	27%	22%	67%	0%	21%
<b>PPV2 (cancer yield)</b>	90%	73%	78%	33%	100%	79%

**PPV1 = Positive Predictive Value for Interpretation**<sup>23</sup>

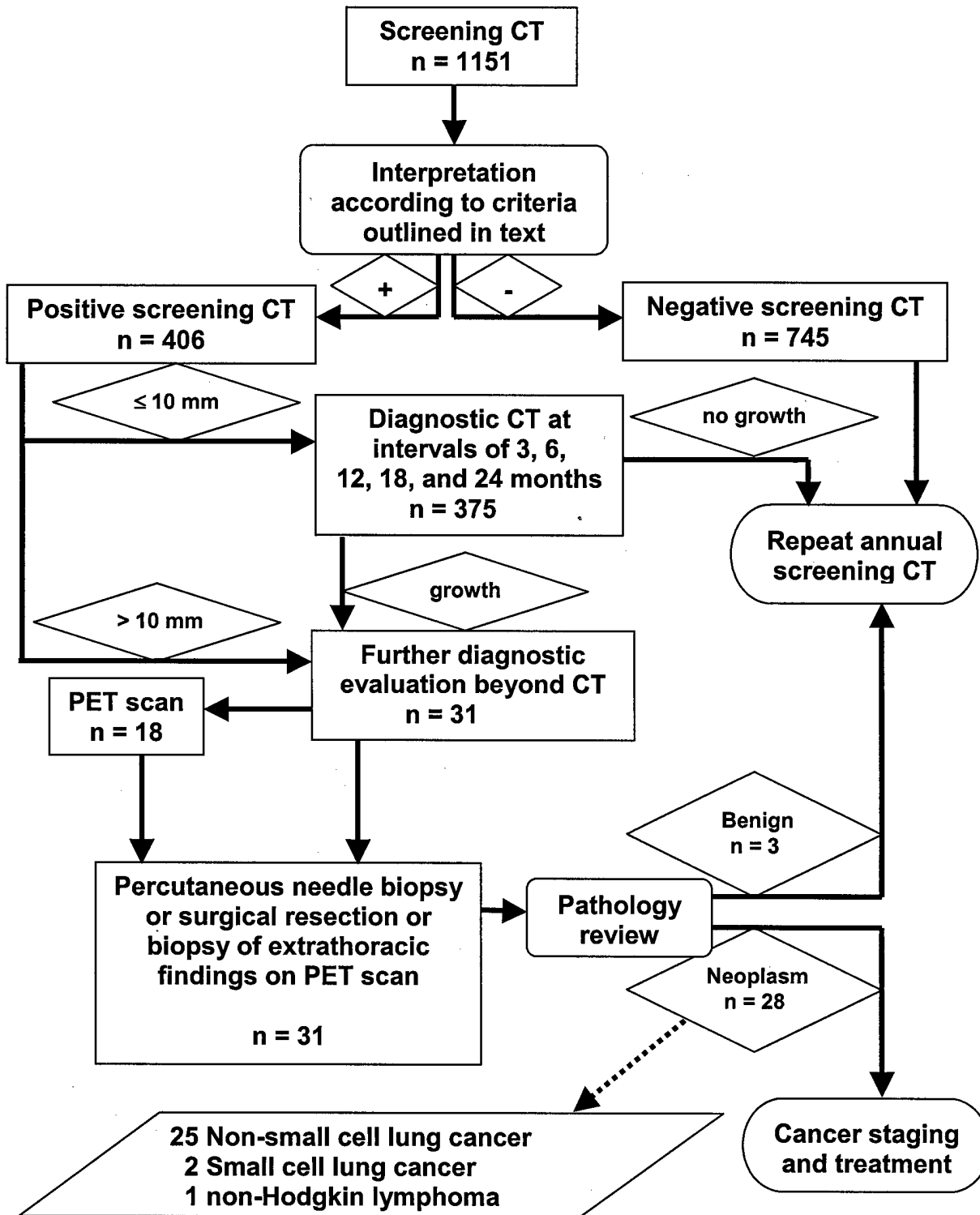
**PPV2 = Positive Predictive Value for Biopsy**<sup>23</sup>

**TABLE 4: Characteristics of screening-detected neoplasms**

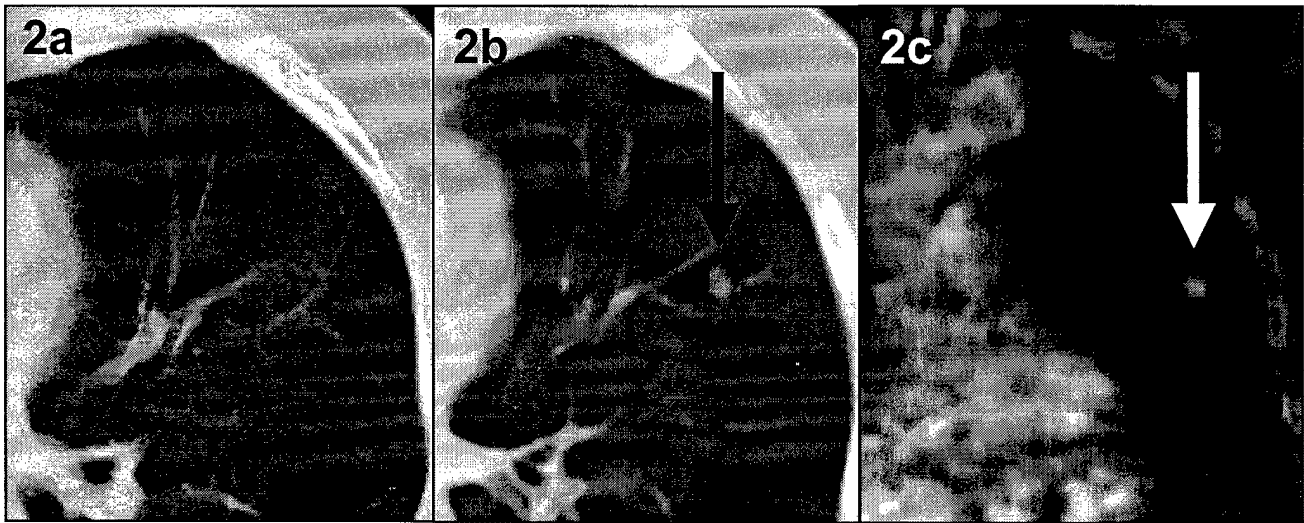
	Number of Prevalence Neoplasms		Number of Incidence Neoplasms		Total	
		%		%		%
<b>All neoplasms</b>	28		18		46	
<b>Lymphoma</b>	1		0		1	2%
<b>Small cell carcinoma - limited stage</b>	2		1		3	7%
<b>Small cell carcinoma - extensive stage</b>	0		2		2	4%
<b>Non-small cell carcinoma</b>	25		15		40	87%
<b>Stages of non-small cell lung cancers</b>						
<b>1A</b>	12	48%	8	53%	20	50%
<b>1B</b>	2	8%	2	13%	4	10%
<b>2</b>	2	8%	3	20%	5	13%
<b>3</b>	4	16%	1	7%	5	13%
<b>4</b>	5	20%	1	7%	6	15%
<b>Cell Types of non-small cell lung cancers</b>						
<b>Adenocarcinoma</b>	14	56%	4	27%	18	45%
<b>Bronchoalveolar carcinoma</b>	6	24%	3	20%	9	23%
<b>Squamous cell carcinoma</b>	2	8%	6	40%	8	20%
<b>Large cell undifferentiated</b>	3	12%	1	7%	4	10%
<b>Carcinoid</b>	0	0%	1	7%	1	3%

**Figure 1: Diagnostic evaluation algorithm for abnormal screening**

CT (prevalence data shown for explanatory purpose)



**Figure 2:** PET for the diagnosis of lung cancer in a screening detected pulmonary nodule; true positive

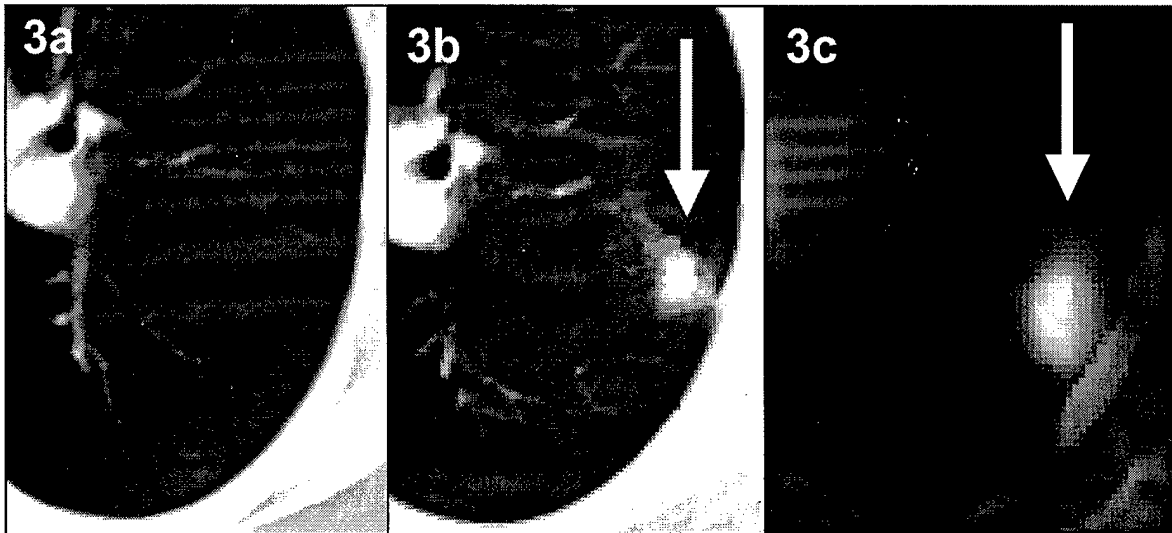


**2a:** Baseline screening CT is negative.

**2b:** Subsequent annual screening CT demonstrates a new circumscribed oval nodule (arrow) 10 mm in diameter.

**2c:** Coronal PET-CT demonstrated metabolic activity with uptake of 18-FDG in the nodule (arrow) for a true diagnosis of pulmonary neoplasm (adenocarcinoma, Stage 1A).

**Figure 3:** PET for the diagnosis of lung cancer in a screening detected pulmonary nodule; false positive

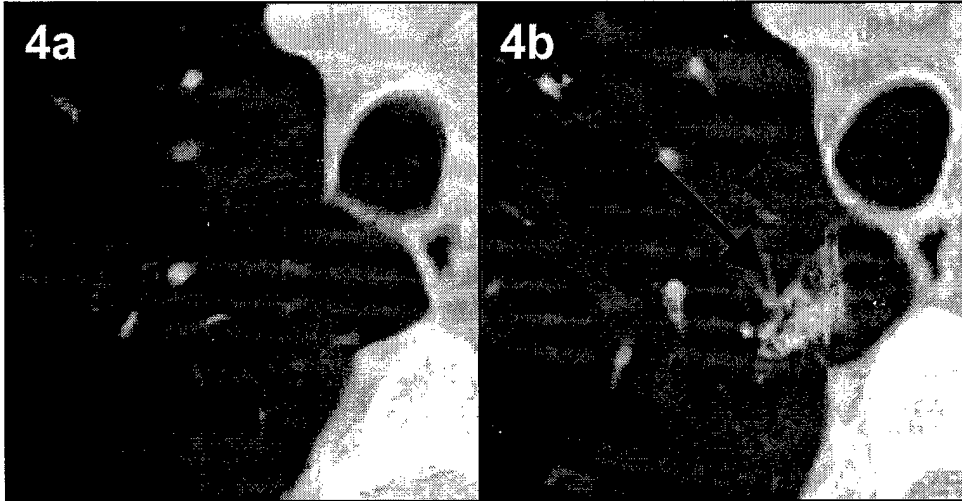


**3a:** Baseline screening CT is negative.

**3b:** Subsequent annual screening CT demonstrates a new irregular nodule (arrow) 14 mm in diameter.

**3c:** Axial PET-CT demonstrated metabolic activity with uptake of 18-FDG in the nodule (arrow) for a false diagnosis of pulmonary neoplasm. Surgery revealed a benign non-caseating granuloma.

**Figure 4:** PET for the diagnosis of lung cancer in a screening detected pulmonary nodule; false negative.



**4a:** Baseline screening CT is negative.

**4b:** Subsequent annual screening CT demonstrates a new ground-glass opacity (arrow) 23 mm in diameter. PET-CT did not demonstrate uptake of 18-FDG, and was falsely negative for the diagnosis of pulmonary neoplasm. Surgery revealed bronchoalveolar carcinoma, Stage 1A.

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**References:**

1. Jemal A, Tiwari RC, Murray T, Ghafoor A, Samuels A, Ward E, Feuer EJ, Thun MJ. Cancer Statistics 2004. *CA Cancer J Clin* 2004; 54: 8-29.
2. Fry WA, Phillips JL, Menck HR. Ten-year survey of lung cancer treatment and survival in hospitals in the United States: a National Cancer Data Base report. *Cancer* 1999; 86: 1897-1876.
3. Mountain CF. Revisions in the international system for staging lung cancer. *Chest* 1997; 111: 1710-1717.
4. Inoue K, Sato M, Fujimura S, et. al. Prognostic assessment of 1310 patients with non-small cell lung cancer who underwent complete resection from 1980 to 1993. *J Thorac Cardiovasc Surg* 1998; 116: 407-411.
5. Rena O, Oliaro A, Cavallo A, Filosso PL, Donati G, DiMarzio P, Maggi G, Ruffini E. Stage 1 non-small cell lung carcinoma: really early stage? *Eur J Cardiothorac Surg* 2002; 21: 514-519.
6. Thoma P, Doddoli C, Thiron X, Ghez O, Payan-Defais MJ, Giudicekki R, Fuentes P. Stage 1 non-small cell lung cancer: a pragmatic approach to prognosis after complete resection. *Ann Thorac Surg* 2002; 73: 1065-1070.
7. Lopez-Encuentra A, Duque-medina JL, Rami-Porta R, Gomez de la Camara A, Ferrando P. Staging in lung cancer: is 3 cm. A prognostic threshold in pathologic stage 1 non-small cell lung cancer? *Chest* 2002; 121: 1515-1520.
8. Wagner H, Ruckdeschel JC. Lung Cancer. In, *Cancer Screening*, Reintgen DS, Clark RA, eds., Chapter 6, pp. 118-149; 1996, Mosby, St. Louis.

- 
9. Fontana RS, Sanderson DR, Woolner LB, Taylor WF, Miller WE, Muhm JR. Lung cancer screening: the Mayo program. *J Occup Med* 1986; 8:746-750.
  10. Tockman M. Survival and mortality from lung cancer in a screened population. *Chest (suppl)* 1986; 89: 324s
  11. Martini N. Results of the Memorial Sloan-Kettering study in screening for early lung cancer. *Chest (suppl)* 1986; 89: 325s
  12. Kubik A, Parkin DM, Khat M, et. al. Lack of benefit from semi-annual screening for cancer of the lung: follow-up report of a randomized controlled trial on a population of high-risk males in Czechoslovakia. *Int J Cancer* 1990; 45: 26-33.
  13. Fontana RS, Sanderson Dr, Woolner LB, et. al. Screening for lung cancer. A critique of the Mayo Lung Project. *Cancer* 1991; 67: 1155-1164.
  14. Miller A. Lung cancer screening: summary. *Chest (suppl)* 1986; 89: 325s
  15. Henschke CI, McCauley DI, Yankelevitz DP, McGuinness G, Miettinen OS, Libby DM, Pasmantier MW, Koizumi J, Alkorki NK, Smith JP. Early Lung Cancer Action Project: overall design and findings from baseline screening. *Lancet* 1999; 354: 99-105.
  16. Sone S, Li F, Yang ZG, Hoda T, Maruyama Y, Takashima S, Hasegawa M, kawakami S, Kubo K, Haniuda M, Yamanda T. Results of three-year mass screening programme for lung cancer using mobile low-dose spiral computed tomography scanner. *Br J Cancer* 2001; 84: 25-32.
  17. Nawa T, Nakagawa T, Kusano S, Kawasaki Y, Sugawara Y, Nakata H. Lung cancer screening using low-dose spiral CT. *Chest* 2002; 122: 15-20.
  18. Sobue T, Moriyama N, Kaneko M, Kusumoto M, Kobayashi T, Tsuchiy R, Kakinuma R, Ohmatsu H, Nagai K, Nishiyama H, Matsui E, Eguchi K. Screening for lung cancer with low-

---

dose helical computed tomography; Anti-Lung Cancer Association Project. *J Clin Oncol* 2002; 20: 911-920.

19. Swensen SJ, Jett JR, Sloan JA, Midthun DE, Hartman TE, Sykes AM, Aughenbaugh GL, Zink FE, Hillman SL, Noetzel GR, Marks RS, Clayton AC, Pairolero PC. Screening for lung cancer with low-dose spiral computed tomography. *Am J Respir Crit Care Med* 2002; 165: 508-513.

20. Diederich S, Wormanns B, Semik M, Lenzen H, Roos N, Heindel W. Screening for lung cancer with low-dose spiral CT: prevalence in 817 asymptomatic smokers. *Radiology* 2002; 222: 773-781.

21. Pastoino U, BellomivM, Landoni C, De Fiori E, Arnaldi P, Picchio M, Pelosi G, Boyle P, Fazio F. Early lung cancer detection with spiral CT and positron emission tomography in heavy smokers: 2-year results. *Lancet* 2003; 362: 593-597.

22. Tockman MS, Anthonisen NR, Wright EC, Donithan MG. Airways obstruction and the risk for lung cancer. *Ann Intern Med* 1987; 106: 512-518.

23. Chirikos T, Hazelton T, Tockman M, Clark RA. Screening for lung cancer with CT: a preliminary cost-effectiveness analysis. *Chest* 2002; 121: 1507-1514.

24. No author. Clinical Practice Guideline Number 13. Quality determinants of mammography. U.S. Department of Health and Human Services. Public Health Service. Agency for Health Care Policy and Research. Rockville, Maryland. AHCPR Publication No. 95-0632. October 1994. pp. 74-86.

25. Linver MN, Osuch JR, Brenner RJ, Smith RA. The mammography audit: a primer for the mammography quality standards act (MQSA). *AJR* 1995;165:19-25.

- 
26. Lardinois D, Weder W, Hany TF, Kamel EM, Korom S, Seifert B, von Schulthess GK, Steinert HC. Staging of non-small-cell lung cancer with integrated positron-emission tomography and computed tomography. *N Engl J Med* 2003; 348: 2500-2507.
  27. Line BR, White CS. Positron emission tomography scanning for the diagnosis and management of lung cancer. *Curr Treat Options Oncol* 2004; 5: 63-73.
  28. Tan BB, Flaherty KR, Kazerooni EA, Iannettoni MD; American College of Chest Physicians. The solitary pulmonary nodule. *Chest* 2003; 23(1 Suppl): 89S-96S.
  29. Stroobants S, Verschakelen J, Vansteenkiste J. Value of FDG-PET in the management of non-small cell lung cancer. *Eur J Radiol* 2003; 45: 49-59.
  30. Fletcher JW. PET scanning and the solitary pulmonary nodule. *Semin Thorac Cardiovasc Surg* 2002; 14: 268-274.
  31. Gould MK, Maclean CC, Kuschner WG, Rydzak CE, Owens DK. Accuracy of positron emission tomography for diagnosis of pulmonary nodules and mass lesions: a meta-analysis. *JAMA* 2001; 285: 914-924.
  32. Heyneman LE, Herndon JE, Goodman PC, Patz EF. Stage distribution in patients with a small ( $\leq 3$  cm.) primary nonsmall cell lung carcinoma. Implication for lung carcinoma screening. *Cancer* 2001; 92: 3051-3055.
  33. Suzuki K, Nagai K, Yoshida J, Nishimura M, Nishiwaki Y. Predictors of lymph node and intrapulmonary metastasis in clinical stage 1A non-small cell lung carcinoma. *Ann Thorac Surg* 2001; 72: 352-356.
  34. Jung KJ, Lee KS, Kim H, Kwon OJ, Shim YM, Kim TS. T1 lung cancer on CT: frequency of extrathoracic metastases. *J Comput Assist Tomogr* 2000; 24: 711-718.

- 
35. Miller DL, Rowlan CM, Deschamps C, Allen MS, Trastek VF, Pairolero PC. Surgical treatment of non-small cell lung cancer 1 cm. Or less in diameter. *Ann Thorac Surg* 2002; 73: 1545-1550.
  36. Patz EF, Rossi S, Harpole DH, Herndon JE, Goodman PC. Correlation of tumor size and survival in patients with stage 1A non-small cell lung cancer. *Chest* 2000; 117: 1568-1571.
  37. American College of Radiology Imaging Network (ACRIN). Contemporary Screening for the Detection of Lung Cancer; ACRIN Protocol A6654; National Cancer Institute grant CA 80098; [http://www.acrin.org/current\\_protocols.html](http://www.acrin.org/current_protocols.html)
  38. Ford LG, Minasian LM, McCaskill-Stevens W, Pisano ED, Sullivan D, Smith RA. Prevention and Early Detection Clinical Trials: Opportunities for Primary Care Providers and Their Patients. *CA Cancer J Clin* 2003; 53: 82-101.
  39. Clark RA, Reintgen DS. Principles of Cancer Screening. In Reintgen DS, Clark RA, (eds.) *Cancer Screening*. Mosby Year Book Publications, St. Louis MO, 1996. Chapter 1, pp. 1-22.
  40. Black WC. Advances in radiology and the real versus apparent effects of early diagnosis. *Eur J Radiol* 27: 116-122, 1998
  41. Soda H, Oka M, Tomita H, et. al. Length and lead time biases in radiologic screening for lung cancer. *Respiration* 66: 511-517, 1999
  42. Black WC. Overdiagnosis: An under-recognized cause of confusion and harm in cancer screening. *J Natl Cancer Inst* 92:1280-1282, 2000
  43. Parkin DM, Moss SM. Lung cancer screening: improved survival but no reduction in deaths – the role of overdiagnosis. *Cancer* 89: 2369-2376, 2000
  44. Kodama K, Higashiyama M, Yokouchi H, et. al. Natural history of pure ground-glass opacity after long-term follow-up of more than 2 years. *Ann Thorac Surg* 73: 386-392, 2002

45. Dammas S, Patz EF Jr., Goodman PC. Identification of small lung nodules at autopsy: implications for lung cancer screening and overdiagnosis bias. *Lung Cancer* 33: 11-16, 2001
46. Reich JM. Improved survival and higher mortality. The conundrum of lung cancer screening. *Chest* 2002; 122: 329-337.