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NLST ACRIN Biomarker Repository Originating from the Contemporary Screening for the Detection of Lung Cancer Trial (NLST, ACRIN 6654):Design, Intent, and Availability of Specimens for Validation of Lung Cancer Biomarkers

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Abstract

Lung cancer continues to be a major public health problem, and more patients die from this disease than any other cancer. The vast majority of patients present with advanced stage disease when therapeutic options are limited and the overall 5 year survival rate remains approximately 15%. Screening with low dose helical computed tomography (CT)has been suggested for early detection, although the effect on mortality is currently under investigation. As part of the National Lung Cancer Screening Trial, a specimen biorepository including blood, sputum, and urine were collected serially for the primary purpose of validating early detection lung cancer biomarkers. In addition tumor samples have been obtained from patients diagnosed with lung cancer to be included in a tissue microarray. This commentary describes the rationale, composition, intent, and availability of specimen in the biorepository.

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Introduction

The National Lung Cancer Screening Trial (NLST) is a cooperative, NCI sponsored randomized trial that was initiated in 2002 with the primary goal to determine if screening for lung cancer with CT, as compared to chest radiographs, reduces lung cancer specific mortality. The trial represented a merger of two NCI-sponsored activities, the NCI Lung Screening Study (LSS) and the American College of Radiology Imaging Network (ACRIN). It enrolled over 50,000 high risk individuals (heavy current cigarette smokers or former smokers) from more than 30 sites across the US, while about one third of participants were accrued by the ACRIN arm (ACRIN 6654). These participants, ages 55–74 years, will be followed for at least five years following enrollment, which was complete in February 2004, to determine if low dose helical CT reduces lung cancer deaths relative to chest radiographs. Details concerning the design of this trial can be found on the NIH-NCI website (http://www.cancer.gov/nlst/what-is-nlst).

As part of this large, unique screening opportunity, biospecimens were collected with the fundamental goal of validating biomarkers that have been carefully tested in pilot data could be used to complement or replace imaging for early detection of lung cancer [1]. Serial blood, sputum, and urine samples were collected using a standardized protocol on approximately 10,300 NLST ACRIN participants. Specifically, the blood and urine samples were collected by the study center at the time of each screening visit, whereas the sputum was collected by the participant at home and shipped by the participant to the central repository. The specimens are stored in a central repository, and linked through a unique participant identifier to extensive clinical data. This report briefly describes the repository, its intent and its availability for investigators interested in biomarker validation studies in early detection of lung cancer.

Repository Description

The repository consists of approximately 107,000 each of buffy coat and plasma samples – participants could provide as many as 12 blood samples (4 vials at each screening), 54,600 urine samples – participants could provide as many as 6 samples (2 vials at each screening), and 36,000 sputum cell pellet specimens – participants could provide as many as 6 samples (2 cups at each screening). Out of approximately 10,300 participants, over 99% provided at least one blood sample (including buffy coat and plasma) and at least one urine sample, and over 74% provided these samples at all 3 screenings. Approximately 80% provided at least one sputum sample to produce cell pellet and about 27% provided sputum samples to produce cell pellet at all 3 screenings. The detailed description of the repository samples will be posted on the ACRIN web site (http://www.acrin.org/TabID/145/Default.aspx.). All participants from whom samples were collected signed an informed consent prior to the prospective collection of samples using a standardized protocol. All participant information was entered into a HIPAA-compliant ACRIN central database.

Tissue specimens from those participants who underwent surgical resection for lung cancer were requested for future inclusion into a tissue microarray (TMA). These specimens included tissue from lung tumor, normal lung tissues, and any tissues from resected metastatic lymph nodes or other sites. At no time was an invasive or dangerous procedure performed solely for the purposes of this trial.

Specimen Collection and Processing

The multi-institutional design of the NLST ACRIN trial necessitated the development of a uniform sample procurement protocol. The University of Colorado provided a biospecimen and processing kit containing the requisite tubes and containers. As in any multi-institutional trial, uniform collection of patient samples is a daunting task, although we tried to develop a

standardized operating procedure within the NLST ACRIN study protocol (http://www.acrin.org/PROTOCOLSUMMARYTABLE/PROTOCOL6654/tabid/145/Default.aspx). The following samples and processing methods were utilized:

1. Blood was drawn from participants into four 8 ml (yellow top) tubes at their yearly visit. Following centrifugation, the plasma and buffy coat were extracted. Buffy coat was aliquoted into four 2-ml citrate (pink top) tubes. Plasma was spun to separate cells and debris, then aliquoted into four 5 ml bar-coded citrate cryovials (orange top). All blood products were immediately stored at −80°C.
2. Urine samples were collected and directly placed into two 5-ml vials for frozen storage.
3. Morning sputum samples were collected by the participants into two sputum cups containing Saccamano's solution. The participants were instructed to cough on three successive mornings into each sputum cup. Mailers were provided for direct mailing of sputum samples to the University of Colorado Specimen Archive. Upon receipt, the sputum samples were pelleted by centrifugation and the cell pellets placed directly in a freezer at −80°C.
4. Based upon review of surgical pathology reports, paraffin blocks containing resected tissues (primary lung cancer, involved lymph nodes and metastases) have been requested retrospectively from the originating pathology centers by the NLST ACRIN sites. The blocks are de-identified, assigned a unique label and forwarded to the UCLA Tissue Array Core Facility. At this laboratory, a single H&E stained slide was prepared and sent to a lung pathologist. The slide is digitized, interpreted, and annotated electronically to identify regions of interest (targets) from which cores for the TMA will be taken. This image is transferred to film, sized to match the original paraffin block, and used by the UCLA TMA laboratory for coring.

Storage Facility and Conditions

The NLST ACRIN biorepository is located in dedicated facilities with state-of-the-art equipment, restricted access and excellent security arrangements. All freezers are under constant monitoring for ventilation and climate control. Quality assurance mechanisms have been constructed and verified by site visits.

Data Collection Forms

A number of case report forms, including specific ones for biomarkers, imaging, and follow-up were collected as part of the screening trial. These forms can be found on the ACRIN website (http://www.acrin.org/Default.aspx?tabid=282). The majority of the forms were completed by the Research Associates (RA) electronically or on paper, although some were completed on paper by the participant and checked by the RA for completeness and legibility. Any questions not understandable to the participant were explained by the RA. The RA reviewed all forms at the time of completion for legibility and completeness, adding any missing data elements. The completed forms are kept in each participant's chart at the site and were entered electronically into the ACRIN web site.

NLST ACRIN Biomarker Repository-Intent

The NLST ACRIN biomarker repository was developed as an integral part of the NLST ACRIN screening trial with the intent to integrate biomarkers into early detection. Blood, sputum, and urine were collected serially as described above, with the primary objective to validate markers that address diagnostic issues complementary to or unresolved by conventional screening

techniques. More specifically, three fundamental areas have been the principal targets of biomarker efforts:

1. To develop biomarkers useful in determining high risk patients

Current criteria used for most screening trials are age and smoking history. This approach defines a large number of individuals at risk for lung cancer, but the overall prevalence of disease even in the highest risk groups is around 1–2% [2–4]. However, individuals in the high risk population often have non-specific imaging findings that require further evaluation, and at least 15–20% of individuals who develop lung cancer are not included in this risk assessment profile [5]. Biomarkers could, in a more efficient manner, help identify which patients are at highest risk for developing lung cancer and should therefore have an imaging study. Because the NLST ACRIN repository contains longitudinal samples, prediction models for developing lung cancer can be tested.

2. To validate biomarkers useful in distinguishing benign from malignant pulmonary nodules detected on imaging studies

Participants in CT screening trials often have non-specific findings, and in some studies, up to 70% will have an indeterminate nodule [2, 4]. The majority of individuals require additional procedures, including CT, positron emission tomography (PET) or an invasive procedure. It has been reported that up to 30% of "highly suspicious" lesions discovered in CT screening trials proved benign at surgery[2, 4, 6]. If a biomarker(s) could immediately determine which patients with an indeterminate pulmonary nodule had lung cancer or no lung cancer, then further evaluation or treatment could be initiated without delay[7]. There would be no need for a wait and watch approach with sequential imaging [8]. Thus some patients would be able to forego follow-up studies or an invasive procedure, while patients with cancer could be treated.

3. To validate biomarker candidates predictive of tumor behavior

Once lung cancer is detected, prognosis and therapy are based on stage at presentation. However, lung tumors are heterogeneous and exhibit a wide spectrum of clinical behavior. This is most notable in patients with early stage disease, as almost 40% will develop metastasis [9]. Currently, there is no way to distinguish between individuals who will develop recurrence and, at the other end of the spectrum, patients with indolent tumors. While the concept of a non-aggressive lung cancer may be difficult to initially understand, the results of CT screening trials as well as autopsy series suggest that non-aggressive tumors will be detected, and thus overdiagnosis bias needs to be considered [2, 10–14]. That is, tumors that would never have affected the patient's natural history are being detected, diagnosed as lung cancer, and removed despite the fact that they are not virulent and would never have become clinically apparent. While these lesions are radiographically and histologically indistinguishable from more aggressive tumors, there is currently no way to determine phenotype. Biomarkers that could accurately characterize lung cancer, suggesting the appropriate targeted therapy (predictive markers), and providing outcome information (prognostic markers), would be invaluable.

Access and Oversight of the Repository

The NLST ACRIN repository is open to any investigator interested in validating lung cancer biomarkers. The application and guidelines are posted on the ACRIN website (http://www.acrin.org/TabID/145/Default.aspx). Two largely separate committees were created to oversee processes and decisions related to the NLST ACRIN biorepository. These committees are composed of experts in a variety of disciplines related to biomarker development, early lung cancer detection, thoracic imaging, molecular diagnostics, trial design, and statistical analysis. The oversight committees are as follows:

1. NLST ACRIN Tissue Bank and Biomarker Oversight Committee (TBBmOC)

This group has been responsible for the oversight and development of policies and procedures that govern the collection, archiving, quality assurance and appraisal of the Biorepository. This group assesses the status of the biorepository and all quality control efforts.

2. NLST ACRIN Research Evaluation Panel (REP) Committee

This independent scientific group is composed of investigators in the biomarker discovery and early detection communities. They have been charged to:

1. Identify the science that would best be served by use of the specimens and correlative data.
2. Develop and oversee policies and procedures for the strategic marketing of this resource to the appropriate scientists.
3. Develop guidelines and forms for proposals requesting use of the specimens.
4. Develop guidelines for the peer-review of proposals requesting biospecimens as well as the terms of fulfillment of specimens and associated data elements.

Process for Evaluation of Biomarkers and Distribution of Samples

An independent peer-review group called the Biospecimen Review Committee has been established by the NLST ACRIN REP to review all proposals requesting the use of NLST ACRIN biospecimens. The review will include members of ACRIN, NLST ACRIN investigators, and *ad hoc* members of the lung cancer scientific community who have expertise in biomolecular technologies and the evaluations under consideration.

Review Criteria for Applications to Use NLST ACRIN Biospecimens

Review criteria are based on scientific merit and compatibility with NLST ACRIN objectives. All NIH review criteria will be applicable, although the following objectives will be strongly considered:

1. Scientific merit
2. Technical parameters: reproducibility, sensitivity, specificity, throughput, automation
3. Clinical or scientific impact
4. Practicality and feasibility; e.g., cost, required sample size and amount of biospecimen required
5. Collaborative strength, including contribution of resources and technology

Application and Review Process

The *ad hoc* committees will review all proposals for access to the biorepository specimens. The submission and review process is described below, and detailed on the ACRIN website (http://www.acrin.org/TabID/145/Default.aspx).

1. A **Pre-Proposal | Letter of Intent** limited to two pages must be submitted six weeks prior to application deadlines. This should include an abstract of the proposal and the name and all contact information of the principal investigator. Validation studies are collaborative, therefore, the pre-proposal must name the NLST ACRIN sites and associated collaborators that will participate in the research.

2. The full proposal should not exceed five pages. A detailed background and rationale are not necessary, but presentation of preliminary data documenting the performance of the proposed marker is mandatory.
3. Copies of proposals received by the designated receipt date are forwarded from ACRIN Headquarters to the members of the constituted Biospecimen Review Committee within one week of the proposal receipt date.
4. Within eight weeks of the receipt date, the Biospecimen Review Committee reviews and scores proposals in a study section teleconference.
5. Results of the review and the evaluations are forwarded to ACRIN Headquarters.
6. The independent NLST Data and Safety Monitoring Board are advised of the approved applications.
7. The NLST ACRIN Executive Committee renders final approval of the reviewed.
8. ACRIN headquarters communicates peer-review decisions and forwards evaluations to the principal investigators.

Structure and Format of Validation Study Proposals

The following items will be requested for the proposal:

- Clinical relationship to early detection or diagnosis.
- Background and significance.
- Preliminary data & methods.
- Data analysis plan.
- Collaborations.
- Future Plans.

Data Analysis, Data Sharing, Chronology of Reporting and Intellectual Property Management

All analyses of the performance characteristics of biomarker assays will be centralized at the ACRIN Biostatistics Center located at Brown University. The clinical data associated with specimens will not be released with the biospecimens until one year following publication of the NLST primary endpoint. After this time, analyses may be collaborative between the investigators and ACRIN Biostatistics Center. This approach ensures the integrity of the trial endpoints and could facilitate the integration of multiple platforms applied on same samples. It is hoped that the complementary nature of different biomarkers will improve diagnostic accuracies and prediction rates.

Institutions involved in NLST ACRIN biomarker validation studies must meet the NIH policies for sharing of data. The NLST ACRIN biomarker initiative is premised on the belief that an established, integrated and multi-disciplinary environment will expedite clinical applications of biomarker validation.

Data sharing guidelines are as follows: Raw data and processed data generated through the proposal on these biospecimen sets will be shared and deposited to NLST ACRIN BDMC. Raw data will be made readily available for research purposes to qualified individuals within the scientific community in accordance with the NIH Grants Policy Statement

(http://grants.nih.gov/grants/policy/nihgps/) and the Principles and Guidelines for Recipients of NIH Research Grants and Contracts on Obtaining and Disseminating Biomedical Research.

The NLST ACRIN Biomarker initiative requires the raw data to be made accessible for further analysis to the larger scientific community within nine months following completion of data analysis. Investigators will exercise intellectual property rights should any be generated through this proposal. Users may collaborate with industry, but the raw data generated on those samples should remain available to the larger community.

Summary

Well-annotated longitudinal samples were uniformly collected and stored in a central repository in the context of a screening trial, with a primary endpoint to determine if screening for lung cancer with low dose helical CT reduces mortality. The NLST ACRIN specimen bank represents a unique opportunity to complement imaging and validate early detection biomarkers for lung cancer. Biomarkers have the potential to improve diagnostic efficiency, reduce cost, and affect the evaluation and management of these patients. While imaging has played an important role in diagnosing, staging and following patients with lung cancer, new strategies using biomarkers offer alternative opportunities to address unresolved diagnostic issues in oncology.

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