

BLOOD REPOSITORY FOR THE VALIDATION OF LUNG CANCER BIOMARKERS

LUNG CANCER BIOMARKERS GROUP

Pierre P. Massion, MD
William L. Bigbee, PhD
Karl Krueger, PhD
Peter Ujhazy, PhD
Jonathan S. Wiest, PhD
Jackie Dahlgren, PhD
Mark Thornquist, PhD
Mel Tockman, MD, PhD
Wilbur Franklin, MD
Jenny Mao, MD
Jim Jett, MD
Bill Rom, MD
Deni Aberle, MD
Chip Petricoin, PhD
Tracey Colpitts, PhD
Sam Hanash, MD, PhD

Abstract:

The Lung Cancer Biomarkers Group (LCBG) consists of scientists from the Early Detection Research Network (EDRN), Lung Cancer Specialized Programs of Research Excellence (SPORes), the NCI and several other researchers. The objectives of the LCBG are to formulate specimen reference sets to be used for testing biomarkers for early detection or diagnosis of lung cancer. At present four reference sets have been planned using serum and plasma samples. The first two reference sets (A and B) are retrospective where select clinically annotated samples currently stored in freezers are provided by six institutions around the country. Collection of two additional reference set (C) is currently in preparation to be collected prospectively from six institutions. Sets A and C (180 cases > 50% Stage I, 180 controls, 75 other cancers) will focus on prevalidation of biomarkers for the diagnosis of lung cancer from patients with an abnormal chest X-ray or at high risk for lung cancer. Set B (38 cases, 87 controls, 25 other cancers) will focus on rapid prevalidation of biomarkers for early detection of lung cancer in the context of computed-tomography (CT) screening of high risk individuals. Patients presenting with suspicious nodules between 0.5 – 3 cm on CT-screening are required for inclusion in set B. All sets will be subdivided into a smaller rapid prevalidation subsets of individual biomarkers, whereas the full sets are required for prevalidation of multi biomarker panels or full validation. These reference sets will be assembled and stored at the NCI facility in Frederick, MD. Any investigator studying promising lung cancer biomarkers can request access to these sets pending approval of your application by an internal review committee within the LCBG.

VALIDATION OF BLOOD BIOMARKERS FOR LUNG CANCER

| | Page # |
|--|---------------|
| Table of Contents: | 2 |
| 1. Background | 3 |
| 2. Goals | 3 |
| 3. Strategy | 3 |
| 4. Biomarker Evaluation | 4 |
| 1 Rapid pre-validation | |
| 2 Panel of biomarker validation | |
| 3 Phase II validation | |
| Controls | |
| Sample collection and standardization | |
| 5. Description of reference serum/plasma sets A, B, and C | 7 |
| 1 Study populations | |
| 2 Cases eligibility | |
| 3 Controls eligibility | |
| 4 Sample size | |
| 5 Institution provider candidates | |
| 6. Sample size justification | 9 |
| 7. Required and desired Common Data Elements (CDEs) | 13 |
| 8. Sample collection and processing requirements | 13 |
| 9. Storage conditions | 14 |
| 10. Process for evaluation of biomarkers and distribution of samples | 15 |
| 1 Review criteria | |
| 2 Review process | |
| 3 Information for pre-validation study proposals | |
| 11. Data analysis, data sharing, chronology of reporting and intellectual property management | 17 |
| 12. IRB approval/ Material Transfer Agreements | 18 |
| 13. Reference sets summary | 19 |
| 13. References | 20 |
| 14. Appendices | |
| A. Repository description | 21 |
| B. Study application form and scientific proposal | 24 |
| C. Reference sample set sharing guidelines | 27 |
| D. Specimen collection Standard Operating Procedure | 31 |
| E. Common Data Elements (CDEs) | |
| 1. Required | 35 |
| 2. Desired | 42 |
| F. Material Transfer Agreement form | 50 |

PREVALIDATION OF SERUM/PLASMA BIOMARKERS FOR LUNG CANCER

1. Background

Early detection of lung cancer is urgently needed in the management of this deadly disease. Despite recent advances in molecular diagnostics, no specific biomarker for the early detection of lung cancer has reached the clinic. New non-invasive discovery approaches for the of early diagnosis of lung cancer have led to the identification of a series of blood based candidate cancer biomarkers (from genomic epigenomic and proteomic approaches) require independent validation in larger sets of well annotated samples from multiple institutions and need to be compared and combined to determine diagnostic accuracy.

2. Goals

The NCI/EDRN/SPORE Lung Cancer Biomarkers Group (LCBG) began its activities back in November 2004 and developed clear objectives and strategies on how to begin validating a series of candidate biomarkers for the early detection of lung cancer. The initial goal of the LCBG is to develop the requisite sample resources to **validate** serum/plasma biomarkers for the early diagnosis of lung cancer. Researchers may use these resources and process for continued biomarker refinement but this is not the primary activity of the LCBG.

Our specific goals include:

1. Develop reference case/control serum/plasma sets held in a NCI repository and make these samples available to the research community.
2. Define, refine and validate blood-based biomarkers for lung cancer.
3. Test reproducibility of biomarkers within and across institutions.
4. Test reproducibility of biomarkers within and across analytical platforms.

3. Strategy

Our strategy is to engage a series of academic institutions from the NCI, EDRN and SPOREs in establishing a “repository” of well annotated blood samples. These blood samples are divided in 3 sets and will be distributed to the scientific community based on their scientific merit.

The first step is to validate the markers based in **existing blood samples** at different institutions, already **collected yet under different protocols (sets A and B)**.

The second step is to validate the markers from different institutions that **will be prospectively collected under identical collection Standard Operating Procedure (set C)**.

The rationale for the 3 sets is described as follows:

REFERENCE SETS A and C focus on pre-validation of biomarkers of *diagnosis of lung cancer* and target lung cancer diagnosed for individuals at high risk for lung cancer or abnormal chest x-ray (CXR) or chest computer tomography (CT) but outside of the context of a CT screening trial. **The clinical question** to be tested after pre-validation relates to whether a serum/plasma biomarker has added value to current clinical tests (CT scan and/or PET scan) for the diagnostic evaluation of pulmonary nodules

and to whether such a biomarker could reduce the number, and the attendant cost, of unnecessary invasive tests (PET or tissue biopsy) or futile thoracotomies.

REFERENCE SET B focus on pre-validation of biomarkers of *early diagnosis (screening) of lung cancer* and targeting a specific population of lung cancer patients diagnosed in the context of a computed tomography (CT)-based screening trial of high risk individuals. **The clinical question** to be tested after pre-validation relates to whether a serum/plasma biomarker has added diagnostic value to current tests (CT scan and/or PET scan) for the diagnostic evaluation of CT-detected pulmonary nodules.

| | Set A | Set B | Set C |
|------------------|----------------------|-----------|-------------|
| Collection | Prospective | | Prospective |
| SOP | No or not unique SOP | | Unique SOP |
| Date | 2000-2005 | | 2006-on |
| Clinical setting | Diagnosis | Screening | Diagnosis |
| Cases | 270 | 86 | 178 |
| Controls | 241 | 147 | 179 |
| Other ca | 67 | 0 | 37 |

The advantage of this study is that all biomarkers can be directly compared with each other on equivalent specimens to determine which biomarker panels perform better or are complementary with other panels. Furthermore, since candidate biomarkers were discovered on similar platforms, this study might help determine if a more effective diagnostic panel can be assembled by combining markers from the different sites. The advantages of comparing the current genomic, proteomic, epigenomic markers and any future biomarkers that come to fruition with the same reference sets are clear cut. The use of this prospective reference set could thus serve as a follow-up for the prevalidation studies of the proteomic markers and may contribute to Phase II validation studies.

A Standard Operating Protocol is being implemented by all centers for collection of the specimens is proposed. Collection will include preparation of serum, plasma and peripheral blood mononucleated cells (PBMC) samples from each individual.

A careful annotation of common data elements (CDEs) has been implemented by the LCBG. Specific lung cancer related CDEs were assembled and a database created at the EDRN Data Management and Coordinating Center (DMCC) at the Fred Hutchinson Cancer Research Center (FHCRC) following the guidance and expertise of the EDRN in this matter (Mark Thornquist and Jackie Dahlgren). Eight participating sites have already begun mapping the CDEs to the DMCC website for a set of required and desired CDEs.

4. Biomarker evaluation

Evaluation of candidate biomarkers is needed in at least 3 specific contexts:

4. 1. *Rapid single biomarker pre-validation set:* A biomarker with demonstrated good performance in a discovery sample set, typically from one institution, needs to be tested first in samples from *independent clinical populations* before going forward to validation in the combined pre-validation set or in a multi-center validation study.

4. 2. *Panel of biomarkers pre-validation set:* The performance of an individual biomarker may be insufficient alone but may have utility when *combined in a panel* with other biomarkers measured in the same sample set. In addition to this use, the combined pre-validation set may also be used in lieu of a multi-center validation study to validate a single marker or to construct and validate a pattern analysis marker (e.g., a proteomics profile), which can be considered to be a single marker with a complex decision rule.

This combined pre-validation set must satisfy two purposes, and hence will have two sets of specimens. In this evaluation, investigators will establish (in the “training set”) a combination marker decision rule(s). In the second set of specimens (the “test” set, see **4. 3.**) they will evaluate the constructed rule(s) in an independent sample of specimens to determine the rule’s operating characteristics (e.g., sensitivity, specificity, positive and negative predictive value). Since all researchers utilizing the combined pre-validation set will be evaluating their markers on essentially identical sets of specimens and contributing the data from their assays to the central database of assay results at the DMCC, over time the reference set will become progressively more valuable as the number of possible marker combinations that may be constructed and tested using this reference set expand.

4. 3. *Phase II biomarker validation set:* for single or combined biomarkers which have passed **4. 1. or 4. 2.**

Controls

Controls should include blood samples from groups of individuals with matched age, sex, race, smoking status and smoking pack year history of smoking to the lung cancer cases. In addition, there is consensus that these controls include samples from subjects with a clinically relevant spectrum non-malignant lung disease and malignant disease from other organ sites.

Sample collection and standardization:

A critical aspect of serum/plasma biomarker validation is to be able to confirm the predictive value of biomarkers beyond the heterogeneity in sample collection, preparation, storage and shipping (1,2). While difficult to achieve in retrospectively assembled reference sets, we will focus on initially obtaining blood samples from institutions where cases and controls were collected, processed, and stored under the same, or closely related, protocol.

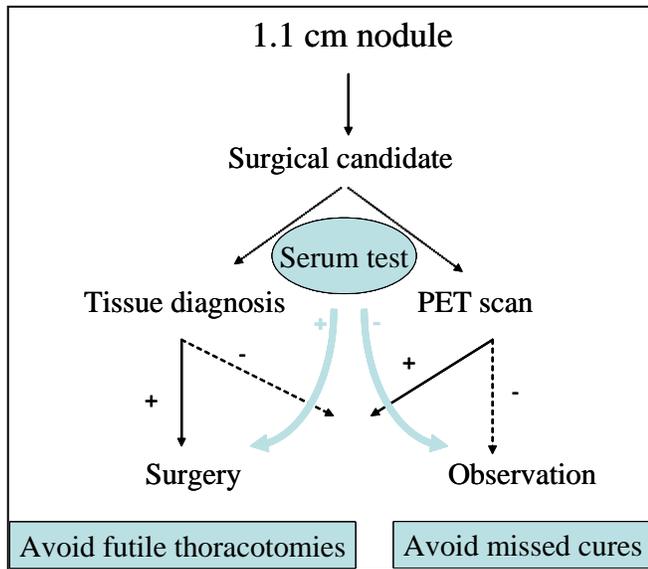


Figure 1. Schematic flowchart illustrating one of the potential uses of a serum/plasma test in the diagnostic approach to lung cancer. A suspicious non-calcified, peripheral nodule of 1.1 cm in diameter is found fortuitously on chest XR or Chest CT. Should the patient be a surgical candidate, the management of this nodule may suggest two clinical options. A PET scan can be obtained and, if found negative, may lead to observation and repeat CT at 3-6 months, accepting the risk of missing the opportunity for early surgical intervention and cure should it really be a malignant lesion. Should the PET scan be positive, the physician may recommend resection. Should the physician decide to obtain a tissue diagnosis (i.e. fine needle aspirate or biopsy), this invasive approach, if confirmatory for cancer, will lead to surgery, and, if negative, may influence the patient and the physician to either observe the lesion accepting the risk of missing a cure, or to undergo surgery and accepting the risk of a futile thoracotomy.

5. Description of reference serum sets

REFERENCE SETS A and C:

1. Clinical setting: Diagnosis of lung cancer

2. Cases eligibility:

- All lung cancers discovered on CXR or on CT
- Pathology: confirmation of malignancy, all histological groups, primary lung cancer
- $\geq 50\%$ Stage I (Stage 1A = T1, N0, M0 and Stage 1B = T2, N0, M0)
- No prior history of lung and other cancer (except for basal cell carcinoma of the skin) in the last 5 years
- Blood collected prior to treatment (chemo/radiation)

3. Controls eligibility

- High risk individuals as defined by ≥ 50 YO, ≥ 30 PKYs of smoking (prevalence of cancer ~1% based on (3-7)) with a lung lesions on CXR or on CT suspicious for lung cancer but proven not to be cancer at 1 year follow up. If at 1 year follow up participant has been diagnosed with a type of cancer other than lung then the participant will be considered an “other cancer” control.
- 75 patients with pathology proven primary cancers from other organ sites (25 breast, 25 colon, 25 prostate) – Set C only 37 other cancers.
- No prior history of lung and other cancer (except for basal cell carcinoma of the skin) in the last 5 years

Cases and controls will be matched for prevalence according to age, sex, race, smoking status, and PKY history of smoking

4. Sample sizes

- (1) Rapid single biomarker pre-validation: 87 cases and 50 controls, 25 other cancer controls
- (2) Panel of biomarkers pre-validation: 150 cases and 150 controls
- (3) Phase II validation: 180 cases and 180 controls, 75 other cancer controls

5. Institution provider candidates

- Pittsburgh (A, B & C)
- Vanderbilt (A & C)
- MDACC (A only)
- UCLA (A and B)
- UCHSC (A only)
- NYU (A, B & C)
- JHU (A only)
- Mayo (B & C)

REFERENCE SET B:

1. Clinical setting

CT screening trial for the early detection of lung cancer

2. Cases eligibility:

- Detected by CT screening

- No prior history of lung or other cancer (except for basal cell carcinoma of the skin) in the last 5 years

- Size: Lung cancer $\geq 0.5\text{cm}$ and $\leq 3\text{cm}$

- Pathology: confirmation of malignancy, all histological groups, primary lung cancer

3. Controls eligibility:

- High risk individuals as defined by ≥ 50 YO, ≥ 30 PKYs of smoking (prevalence of cancer ~1%) undergoing screening chest CT with a lung nodule $\geq 0.5\text{cm}$ and $\leq 3\text{cm}$, free of cancer at the 1 year F/U CT. If at 1 year follow up participant has been diagnosed with a type of cancer other than lung then the participant will be considered an “other cancer” control.

- High risk individuals as defined by ≥ 50 YO, ≥ 30 PKYs of smoking (prevalence of cancer ~1%) undergoing screening chest CT without a lung nodule, free of cancer at the 1 year F/U CT. If at 1 year follow up participant has been diagnosed with a type of cancer other than lung then the participant will be considered an “other cancer” control.

- 25 patients with other cancers (breast, colon, prostate)

- No prior history of lung or other cancer (except for basal cell carcinoma of the skin) in the last 5 years

Match prevalence according to age, sex, race, smoking status, and PKY history of smoking

4. Sample size

- (1) Rapid single biomarker pre-validation: 38 cases and 87 controls, 25 other cancer controls
- (2) Panel of biomarkers pre-validation: insufficient number of samples available
- (3) Phase II validation: insufficient number of samples available

5. Institution provider candidates

Pittsburgh
UCLA
NYU
Mayo

6. Sample size justification

Although the screening contexts for Reference Sets A/C and B have been specified, there are still many different conditions (sensitivities, specificities, and subpopulations) within those contexts in which a potential biomarker may be useful. To calculate sample sizes, we have identified conditions under which a potential marker has definite utility and determined the sample size to have the desired characteristics (power for a given type I error rate) in that condition. The choice of conditions and resulting test statistics used for the power analyses are not intended to place any restriction on the proposals for use of the specimens. Researchers applying for samples from the Reference Sets may anticipate different conditions and propose different test statistics than those assumed in these sample size calculations. However, as part of the application, we expect researchers to specify their analysis plan, state the conditions under which the marker will be considered to have successfully passed validation, and determine the power of their analysis given the numbers of samples in the requested Reference Set(s).

6.1 Rapid single biomarker pre-validation sets

For Reference Sets A/C, the consensus of the clinicians in the LCBG was that a conservative estimate of the prevalence of disease in the screening population would be 1%. There was also consensus that a marker with a sensitivity of 0.80 and specificity of 0.70 would be worthwhile enough to merit further consideration, at least in the context of being included in a panel of markers evaluated in a combined pre-validation specimen set. To merit further consideration as a single marker, the CLCBG thought that a high sensitivity, say 0.85, was needed given the severity of the disease.

If considering all individuals at risk for lung cancer, the positive predictive value (PPV) would be 0.01, the same as the prevalence. For a biomarker to pass the rapid pre-validation phase, it will need to increase the PPV by an amount that is clinically significant. To compute the needed sample size, we specify two PPVs:

- PPV_1 is a PPV that, although better than 0.01, is not sufficiently better than 0.01 to be of clinical utility; we will choose a sample size that will accept such a PPV to move forward with low probability.
- PPV_2 is a PPV that is sufficiently better than 0.01 that it would have definite clinical utility; we will choose the sample size that will accept such a PPV to move forward with high probability.

For the computations here, we choose PPV_1 to be 0.013. PPV_2 is calculated using sensitivity of 0.80, specificity of 0.70, and disease prevalence of 0.01, and is equal to 0.026. Thus, in the screening population we conservatively assume that one in 100 individuals screened will have lung cancer. The consensus of the LCBG members is that a test that increases that frequency to one in 40 screenees would be clinically worthwhile as a possible component in a marker panel for detection of lung cancer. A test that increases the frequency to one in 80 screenees would not be a large enough gain to be clinically worth further investigation as a candidate marker in a panel of markers. The specific combinations of sensitivity and specificity that we will design our sample size around are therefore:

| | Sensitivity | Specificity | PPV | NPV |
|---------------------------|-------------|-------------|-------|-------|
| Definite clinical utility | 0.80 | 0.70 | 0.026 | 0.997 |
| Insufficient sensitivity | 0.40 | 0.70 | 0.013 | 0.991 |
| Insufficient specificity | 0.80 | 0.40 | 0.013 | 0.995 |
| No screening test | -- | -- | 0.010 | 0.990 |

To compute the needed sample size of cases, we determine the sample size for the exact test of a binomial proportion with the property that the observed sensitivity either does not differ from the insufficient sensitivity level (0.40) at a p-value of p_1 or does not differ from the definite clinical utility sensitivity level (0.80) at a p-value of p_2 , but not both. We choose the number of controls in a similar manner using the desired specificity. Intuitively, this means that either the marker result might be no better than a marker of little clinical utility (and hence it's not a good candidate to move forward) or the marker result might be as good as a marker of definite clinical utility (and hence should be moved forward to the next step in validation). The values for p_1 and p_2 are chosen to have the desired power for PPV_1 and PPV_2 . We use $p_1 = 0.025$ and $p_2 = 0.025$. With these values, the needed sample sizes are 27 cases and 50 controls for Reference Sets A/C.

For Reference Set B, in five lung cancer screening trials(3-7), the prevalence of lung cancer among screened individuals with an abnormal CT result ranged from 2.8% to 11.5% , with only the Henschke study showing a rate of greater than 5%. To compute the positive predictive value (PPV) of markers in the screening setting, we will assume a prevalence of disease of 5%. The PPV of CT alone is thus 0.05. The LCBG agreed that in this screening context, a sensitivity of 0.80 and specificity of 0.70 (with resulting PPV of 0.123) had sufficient clinical utility, at least for inclusion in a panel of markers. For a stand-alone marker, a sensitivity of at least 95% was thought necessary. A PPV of 0.075 was deemed not sufficiently better than CT alone to merit further evaluation. Thus, we compute sample size to distinguish among the following three scenarios:

| | Sensitivity | Specificity | PPV | NPV |
|---------------------------|-------------|-------------|-------|-------|
| Definite clinical utility | 0.80 | 0.70 | 0.123 | 0.985 |
| Insufficient sensitivity | 0.46 | 0.70 | 0.075 | 0.961 |
| Insufficient specificity | 0.80 | 0.48 | 0.075 | 0.978 |

Using the same method of determining sample size as discussed for Reference sets A/C, we calculate that we need 38 cases and 87 controls in Reference Sets B.

6.2 Panel of biomarkers pre-validation set

This set will be used to **construct** and **test** a panel of biomarkers for diagnosis of lung cancer. We presume that any individual marker alone will not have adequate performance to merit a multi-center validation study. To construct and test a panel of markers, EDRN and Lung Cancer SPORE collaborators will select a list of biomarker candidates. The assays for these candidates will be performed on the training set and the diagnostic rule will be established. That rule, if it meets the criterion for access (that means the completion of Phase I (8)), will then be validated on the validation set. Validation of individual markers using these sets may skip this first step if there is sufficient evidence, as agreed upon by the EDRN and Lung SPORE collaborators, that they meet the criteria for access. The confirmation on the validation set represents a successful completion of Phase II validation (if the assays are sufficiently developed to be robust and can be used in clinical setting). The sample size required here is much bigger than that for rapid pre-validation because of the requirement to demonstrate sufficient sensitivity and specificity to satisfy Phase II validation.

Sample size and power calculations are not applicable for the classifier **construction** phase (training sample) because the power depends on the signal to noise ratio, the nature of the data and analytical method. The sample size of 150 cases and 150 controls is based on considerations of feasibility and cost, and the fact that this size is as large or larger than most of the biomarker studies reported.

6.3. Phase II validation set

- *For Reference Sets A/C*, the power calculation for the **validation** phase is based on the following assumptions:
 - 1) Given the severity of lung cancer, we assume that a marker must have sensitivity of at least 0.80 and specificity of at least 0.80 to have clinical utility, so a marker with sensitivity or specificity below these thresholds would fail to pass this threshold. We choose our sample size so that a marker with sensitivity above 0.90 and specificity above 0.90 would have high probability of passing the validation step.
 - 2) Power is calculated based on joint tests for sensitivity and specificity and adjusted for this multiplicity. We utilized two-sided tests of significance at a p-value of 0.05 and require approximately 90% power to distinguish the alternatives described in 1).

With these assumptions, the sample size needed is 180 cases and 180 controls. The positive and negative predictive values for the scenarios described above are shown below, under assumptions that the prevalence of lung cancer in the screened group is 0.01 (the conservative estimate used for the rapid pre-validation set).

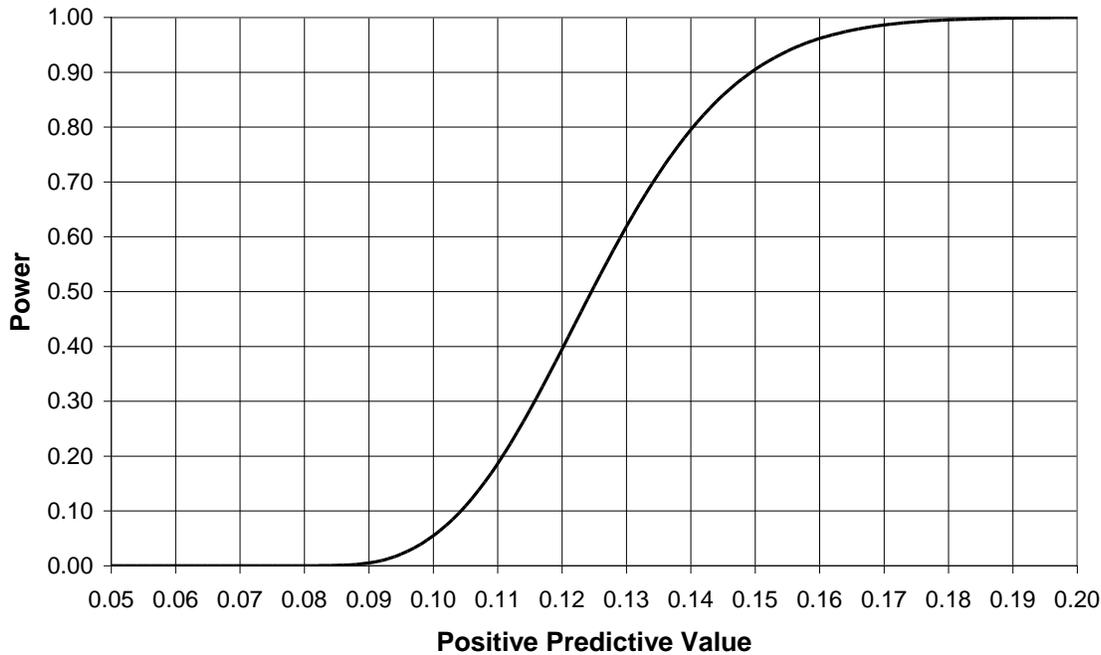
| | Prevalence = 0.01 | | | |
|---------------------------|-------------------|-------------|-------|--------|
| | Sensitivity | Specificity | PPV | NPV |
| Definite clinical utility | 0.90 | 0.90 | 0.083 | 0.9989 |
| Insufficient sensitivity | 0.80 | 0.90 | 0.075 | 0.9978 |
| Insufficient specificity | 0.90 | 0.80 | 0.043 | 0.9987 |

- *For Reference Sets B*, the power calculation for the **validation** phase is based on the following assumptions:
 - 1) We assume that a marker that, for some cutpoint, has sensitivity of 50% and specificity of 85%, with resulting positive predictive value of 0.15, has clear clinical benefit, while a marker with sensitivity of 40% and specificity of 75%, and resulting PPV of 0.078, is clinically unacceptable. When calculating PPV and NPV, we assumed that in the screening population, the prevalence of lung cancer is 0.05.
 - 2) Power is calculated based on joint tests for sensitivity and specificity and adjusted for this multiplicity. We utilized two-sided tests of significance at a p-value of 0.05 and require approximately 90% power to distinguish the alternatives described in 1).

With these assumptions, the sample size needed is 250 cases and 170 controls. Figure 4 demonstrates a sample power curve as a function of PPV for this sample size (it is only a sample since the power depends not just on PPV but also on the combination of sensitivity and specificity).

Figure 4

Sample Power Curve as a Function of PPV



The entire combined pre-validation Reference Set (either A/C or B) may also be used in lieu of a validation study if a marker or panel of markers already has a decision rule developed on independent data and the investigator wishes to validate the marker on the standard specimen reference set. In this case, the marker would be evaluated on both the training and test sets together with the pre-chosen decision rule and the entire combined set being considered a test set. Thus, for Reference Sets A/C, there would be 330 cases and 330 controls analyzed, while for Reference Sets B there would be 320 cases and 400 controls analyzed. The table below shows the differences in sensitivity or specificity detectable using these sample sizes.

Sensitivity/specificity detectable with 90% power

| Sample size | “Bad sensitivity/specificity” | | | |
|-------------|-------------------------------|------|------|------|
| | .800 | .850 | .900 | .950 |
| 320 | .876 | .917 | .954 | .986 |
| 330 | .875 | .916 | .953 | .985 |
| 400 | .869 | .910 | .949 | .983 |

7. Required and desired Common Data Elements (CDEs)

The EDRN has developed Common Data Elements (CDEs) for use in EDRN studies to enhance the ability of studies to share information through the use of identical data elements. It is recognized that retrospective specimens included in these reference serum/plasma sets from different institutions most likely will not have been collected using these EDRN CDEs. For this reason, not all of the established EDRN Core CDEs will be required for the specimens banked in this protocol. **Appendix E** indicates the required and desired CDEs to be collected to annotate the individual from whom a specimen was obtained and indicates the CDEs (all required) needed to annotate the specimen itself. In the event the specific EDRN CDEs were not collected, the EDRN DMCC will work with the investigators at each contributing institution to map their data elements to the respective EDRN CDEs. Once this mapping is completed, the site providing specimens is then responsible for providing the requisite data, stored using the EDRN CDE variable names and formats.

8. Sample collection and processing requirements

- Approximately equal numbers of cases and controls will be included from each of the contributing institutions when possible
- All samples collected contemporaneously at all sites within the past 5 years (since 2000)
- Sample collection: for samples already collected, a copy of the collection protocol is requested from each participating institution. For prospectively collected cases and controls please refer to the suggested Standard Operating Procedure (**Appendix D**)
- Shipping of minimum of 1-2 mL of serum and plasma
- Shipping of sample after FIRST freeze (meaning sample was frozen once at each institution and shipped frozen. Samples will be thawed and aliquoted at the Frederick site.

Aliquots: 8 x 100 μ L and 8 x 25 μ L.

Group any samples that go to different sets and label the box according to which set they belong (Set A, serum / Set A, plasma / Set B, serum / Set B, plasma). Ship samples on dry ice priority overnight delivery to the NCI Frederick Facility and have their FEDEX account number charged for shipping ([2649-0814-0](tel:2649-0814-0)). Address the package to:

Karon Drew
Fisher BioServices
4600 Wedgewood Blvd., Suite H
Frederick, MD 21703

It is best to contact Karon Drew before sending samples. She can be reached at 301-694-5911 and her email address is karon.drew@thermofisher.com. She needs to be notified before samples arrive so that she is prepared to receive and process them. On the shipping package in the Customer Reference field indicate that these samples are for the “EDRN/SPORE Lung Sets”. Inform Karon Drew of the tracking number after the package has been sent out.

Recommended packages for shipping can be purchased at <http://www.saftpak.com/> If undamaged, these containers can be shipped back to the contributing institution. Sufficient dry ice should be enclosed to last for 2 days. Shipping should be done on a Monday or Tuesday to guarantee delivery to the Frederick facility before Friday of the same week.

9. Storage conditions

Consensus on a repository at NCI-Frederick:

The LCBG agreed on initially developing four serum/plasma reference sets (Reference Sets A/C and B]. Tissue samples and other biological specimens will be addressed in the future after showing feasibility with blood samples.

Centralization of the 1-2 mL serum/plasma samples at the NCI Frederick facility:

For oversight and monitoring Fisher BioServices at the NCI Frederick Facility provides as part of the monthly maintenance cost for the freezer the following services: They ensure contents of the freezer will be kept at -80°C and have sufficient backup freezers and power backup to guarantee this. Karl Krueger (NCI/CBRG) will be the chief NCI/LCBG contact for the repository samples, but in his absence Peter Ujhazy (NCI/SPORE) and Sudhir Srivastava (NCI/CBRG) can also contact Fisher BioSciences for shipping of Reference Set samples, i.e. who can have access to which samples given approval by the LCBG designated Specimen Sharing Committee.

10. Process for evaluation of biomarkers and distribution of samples

The *Specimen Sharing Committee* was created within the LCBG and has drafted a process through which the Reference Sample Sets could be accessed (**Appendix C**). This Committee is charged with review of requests for all of the Reference Sample Sets. Common guidelines and procedures were developed. This Committee includes: Drs. Wiest (Chair), Ujhazy, Krueger, Rom, Thornquist and three investigators external to the LCBG to be announced.

1. Review criteria:

Review criteria are based on scientific merit and compatibility with LCBG objectives. Six formal criteria are used to assess the suitability of proposals for access to aliquots of the Reference Sample Set(s):

- Scientific merit
- Study design: relevance of the retrospective Reference Sets A and B and/or prospective Reference Set C
- Technical parameters: reproducibility, sensitivity, specificity, throughput, automation
- Clinical or scientific impact: e.g., more common cancers or a significant impact in less common neoplasia
- Practicality and feasibility: e.g., cost, required sample size, amount of biospecimen required
- Collaborative strength, including contribution of resources and technology.

2. Review process:

The LCBG Specimen Sharing Committee will review all applications for access to the Reference Sample Sets.

The review process is described below:

1. Copies of proposals received by the receipt date are forwarded from the LCBG Program Office to the members of the Specimen Sharing Committee within a week after the application receipt date (web-based electronic review system).
2. The LCBG Specimen Sharing Committee evaluates and scores applications and sends results of the review to the LCBG. The evaluation is expected to be complete within one month following the application receipt date.
3. The LCBG renders final approval by majority vote of the reviewed proposals and communicates decisions to the NCI Frederick Facility for release of the appropriate samples. These actions are expected to occur within three months after the application receipt date.
4. When an application is approved, the investigator will be asked to provide the LCBG a one page abstract describing the study. This abstract will be posted at the website where all information regarding the LCBG reference sets is maintained. The name of the PI, the Institution, Title of Project (do not exceed 81 characters, including spaces and punctuation.) and Abstract (do not exceed 350 words) should be submitted to Jonathan Wiest (wiestj@mail.nih.gov) or Karl Krueger (kruegerk@mail.nih.gov).

3. Additional information for validation study proposals:

Progress of a biomarker to a full validation study is a critical step in the development of a biomarker and is, therefore, a critical priority of the LCBG.

Proposal: See **Appendix B**

A pre-proposal/letter-of-intent, of 1 to 3 pages, must be submitted one month before an application deadline (see **Appendix C**, review process) to the LCBG Program Office,

Jonathan S. Wiest
Laboratory of Cellular Carcinogenesis and Tumor Promotion, 31/4A48
National Cancer Institute
Bethesda, MD 20892.
(wiestj@mail.nih.gov)

Full proposals are reviewed monthly by the Specimen Sharing Committee. Submissions received by the LCBG Program Office by the first of the month are reviewed within 1-2 months by the LCBG.

Recipients of LCBG reference sets will be blinded to the samples unless prior arrangements are made justifying why some samples should be unblinded prior to starting the study. Recipients will need to agree to submit raw data of their analysis to the EDNRN DMCC within four months after receiving the samples at which point they will be unblinded. The EDNRN DMCC agrees not to release recipients' data to anyone outside the DMCC analytic team until after a 3 month interval has passed. The LCBG reserves the right to post the data to a public website at 12 months after the unblinded results have been provided to the investigator providing sufficient time for the investigators to publish their data.

Results of the pre-validation study will be made available to the LCBG for review and comparative/combined analysis with data from other biomarker pre-validation studies using the same Reference Set samples. This process includes a requirement for submission by the investigators of their primary data for confirmatory analysis by the EDNRN DMCC. Details of these guidelines are provided in **Appendix C**.

11. Data analysis, data sharing, chronology of reporting and intellectual property management

Data analysis will primarily occur in the context of the proposal of individual investigators. In addition, a centralized data analysis core group of investigators (yet to be determined) is being discussed and would take full advantage of multiple platforms applied on same samples. It is hoped that the complementary nature of different biomarkers will improve diagnostic accuracies and prediction rates.

Resources sharing guidelines are described in **Appendix C**. Institutions involved in the LCBG intend to meet the NIH policies for sharing of data. The LCBG is premised on the belief that an established integrated, multi-disciplinary environment will expedite clinical applications of biomarker validation.

Data sharing guidelines are proposed as such: Raw data and processed data generated through the proposal on these LCBG reference sample sets will be shared and deposited to a yet to be defined data repository. 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.

Chronology of reporting requirements is still being discussed. The LCBG requires the raw data to be made accessible for further analysis to the larger scientific community within one year after NCI Frederick has delivered the samples to a given investigator.

Investigators will exercise intellectual property rights should any be generated through this proposal, while making such research resources available to the broader scientific community one year after samples are distributed. LCBG members may collaborate with industry but the raw data generated on those samples should remain available to the larger community in the time frame proposed (one year). It is hoped that validated biomarkers may ultimately be commercialized into diagnostic products for early detection of cancer.

12. IRB/Material Transfer Agreement (MTA)

For investigators providing samples to the Reference Sample Sets in the repository:

Copy of the consent form and copy of the protocol with approval date.

- Samples collected with IRB approved consent form.
- Consent informs individuals that the samples will be shared with other investigators and to investigate eventually other types of cancer and other disease
- None of the 18 HIPAA identifiers will be shared, all information is de-identified.

For investigators requesting access to Reference Sample Sets in the repository

IRB approval for use of reference set samples from the repository

Material Transfer Agreement (MTA):

Proposed format: A template MTA is provided in **Appendix F**. Each institution will likely revise this agreement according to their specific needs.

13. Reference sets summary

| | Reference Sets A and C | Reference Set B |
|--|---|---|
| Clinical question | Diagnosis of lung cancer | Early diagnosis of lung cancer |
| Study design | Case-control study | Case-control study |
| Study population | All suspicious lung lesions on CXR or on CT | CT screening |
| Cases | Lung cancers, $\geq 50\%$ Stage I | Detected by CT- Lung cancer $\geq 0.5\text{cm}$ and $\leq 3\text{cm}$ |
| Controls | High risk individuals as defined by ≥ 50 YO, ≥ 30 PKYs of smoking All lung lesions on CXR or on CT suspicious for lung cancer but proven not to be cancer at 1 year follow up 75 patients with other cancers (25 breast, 25 colon, 25 prostate) | Detected by CT with a lung nodule $\geq 0.5\text{cm}$ and $\leq 3\text{cm}$ CT screened without a lung nodule (10% of total # of controls) All free of cancer at the 1 year F/U CT 75 patients with other cancers (25 breast, 25 colon, 25 prostate) |
| Matching criteria | Age, sex, smoking status, PKYs | Age, sex, smoking status, PKYs |
| Sample size | | |
| (1) Single biomarker pre-validation | (1) 27 cases 50 controls \pm 25 other cancer controls | (1) 38 cases 87 controls \pm 25 other cancer controls (plasma only) |
| (2) Panel of biomarkers pre-validation | (2) 150 cases and 150 controls | (2) 150 cases and 150 controls (not available) |
| (3) Phase II validation | (3) 180 cases and 180 controls + 75 other cancer controls (Set C only 37 other cancer controls) | (3) 250 cases, 170 controls + 75 other cancer controls (not available) |
| Pulmonary diseases | Enriched across whole population | Enriched in controls with CT detected lung nodules |
| Institution provider candidates | Pittsburgh (A, B & C) Vanderbilt (A & C) MDACC (A only) UCLA (A and B) UCHSC (A only) NYU (A, B & C) JHU (A only) Mayo (B & C) | Pittsburgh UCLA NYU Mayo |

14. References

- (1) Semmes OJ, Feng Z, Adam BL, Banez LL, Bigbee WL, Campos D, et al. Evaluation of serum protein profiling by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry for the detection of prostate cancer: I. Assessment of platform reproducibility. *Clin Chem* 2005;51:102-12.
- (2) Grizzle WE, Adam BL, Bigbee WL, Conrads TP, Carroll C, Feng Z, et al. Serum protein expression profiling for cancer detection: validation of a SELDI-based approach for prostate cancer. *Dis Markers* 2003;19:185-95.
- (3) Henschke CI, McCauley DI, Yankelevitz DF, Naidich DP, McGuinness G, Miettinen OS, et al. Early Lung Cancer Action Project: overall design and findings from baseline screening. *Lancet* 1999;354:99-105.
- (4) Swensen SJ, Jett JR, Sloan JA, Midthun DE, Hartman TE, Sykes AM, et al. Screening for lung cancer with low-dose spiral computed tomography. *Am J Respir Crit Care Med* 2002;165:508-13.
- (5) Sobue T, Moriyama N, Kaneko M, Kusumoto M, Kobayashi T, Tsuchiya R, et al. Screening for lung cancer with low-dose helical computed tomography: anti-lung cancer association project. *J Clin Oncol* 2002;20:911-20.
- (6) Sone S, Li F, Yang ZG, Honda T, Maruyama Y, Takashima S, et al. 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.
- (7) Pastorino U, Bellomi M, Landoni C, De Fiori E, Arnaldi P, Picchio M, et al. Early lung-cancer detection with spiral CT and positron emission tomography in heavy smokers: 2-year results. *Lancet* 2003;362:593-7.
- (8) Sullivan Pepe M, Etzioni R, Feng Z, Potter JD, Thompson ML, Thornquist M, et al. Phases of biomarker development for early detection of cancer. *J Natl Cancer Inst* 2001;93:1054-61.

15. Appendices**Appendix A. Repository description**

| Serum | | | | | | | | | |
|---|----------|-----------|------------|----------------|-----------|------------|----------|------------|------------|
| | CO | JHH | Mayo | MD Anderson | NYU | UPCI | UCLA | Vanderbilt | Total |
| Set A Cases: Lung Cancer-1 | 0 | 19 | 0 | 23 | 27 | 64 | 0 | 71 | 204 |
| Set A Controls: High Risk-3 | 0 | 0 | 0 | 23 | 26 | 59 | 0 | 72 | 180 |
| Set A Controls: Other Cancers-5 | 0 | 0 | 0 | 0 | 0 | 43 | 0 | 0 | 43 |
| Total | 0 | 19 | 0 | 46 | 53 | 166 | 0 | 143 | 427 |
| Set B Cases: Lung Cancer-6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Set B Controls: High Risk with nodule-8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Set B Controls: High Risk with no nodule-9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Set B Controls: Other Cancers-10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Set C Cases: Lung Cancer-21 | 0 | 0 | 63 | 0 | 32 | 13 | 0 | 70 | 178 |
| Set C Controls: High Risk (prospective)-23 | 0 | 0 | 23 | 0 | 34 | 60 | 0 | 62 | 179 |
| Set C Controls: Other Cancers-25 | 0 | 0 | 27 | 0 | 0 | 0 | 0 | 10 | 37 |
| Total | 0 | 0 | 113 | 0 | 66 | 73 | 0 | 142 | 394 |

| Plasma | | | | | | | | | |
|---|-----------|-----------|------------|----------------|-----------|------------|-----------|------------|--------------|
| | CO | JHH | Mayo | MD Anderson | NYU | UPCI | UCLA | Vanderbilt | Total |
| Set A Cases: Lung Cancer-1 | 42 | 21 | 0 | 0 | 27 | 43 | 20 | 71 | 224 |
| Set A Controls: High Risk-3 | 41 | 0 | 0 | 0 | 26 | 42 | 20 | 70 | 199 |
| Set A Controls: Other Cancers-5 | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | 24 |
| Total | 83 | 21 | 0 | 0 | 53 | 109 | 40 | 141 | 447 |
| Set B | | | | | | | | | |
| Set B Cases: Lung Cancer-6 | 0 | 0 | 37 | 0 | 14 | 35 | 0 | 0 | 86 |
| Set B Controls: High Risk with nodule-8 | 0 | 0 | 25 | 0 | 3 | 25 | 13 | 0 | 66 |
| Set B Controls: High Risk with no nodule-9 | 0 | 0 | 25 | 0 | 22 | 25 | 9 | 0 | 81 |
| Set B Controls: Other Cancers-10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 0 | 0 | 87 | 0 | 39 | 85 | 22 | 0 | 233 |
| Set C | | | | | | | | | |
| Set C Cases: Lung Cancer-21 | 0 | 0 | 63 | 0 | 32 | 13 | 0 | 70 | 178 |
| Set C Controls: High Risk (prospective)-23 | 0 | 0 | 23 | 0 | 34 | 60 | 0 | 62 | 179 |
| Set C Controls: Other Cancers-25 | 0 | 0 | 27 | 0 | 0 | 0 | 0 | 10 | 37 |
| Total | 0 | 0 | 113 | 0 | 66 | 73 | 0 | 142 | 394 |

Appendix B. Study application form and scientific proposal

<http://ccrod.cancer.gov/BiomarkerRequest/>

Appendix C. Reference Sample Set Sharing Guidelines

NCI/EDRN/SPORE Lung Cancer Biomarkers Group Reference Sample Set Sharing Guidelines

The goal of the NCI/EDRN/SPORE Lung Cancer Biomarkers Group (LCBG) is to develop the requisite sample resources to pre-validate serum biomarkers for the early diagnosis of lung cancer. These sample sets will be maintained at the NCI-Frederick repository. Researchers may use these resources and process for testing the reproducibility of biomarkers across institutions and analytical platforms. The Specimen Sharing Committee was created within the LCBG and has designed an application and review process through which these Reference Set samples could be accessed. This Committee is charged with review of requests for either or both of the Reference Set samples. Common guidelines and procedures are being developed and implemented. Applications for multiple Reference Set samples will be considered based on the scientific merit of the application.

The patient population context

1. *Reference Sets A and C* will focus on pre-validation of biomarkers of *diagnosis of lung cancer* and target lung cancer diagnosed for individuals at high risk for lung cancer or abnormal chest x-ray CXR or chest CT but outside of the context of a CT screening trial. **The clinical question** to be tested after pre-validation relates to whether a serum/plasma biomarker has added value to current clinical tests (CT scan and/or PET scan) for the diagnostic evaluation of pulmonary nodules and to whether such a biomarker could reduce the number, and the attendant cost, of unnecessary invasive tests (PET or tissue biopsy) or futile thoracotomies.

2. *Reference Set B* will focus on rapid pre-validation of biomarkers of *early diagnosis (screening) of lung cancer* and targeting a specific population of lung cancer patients diagnosed in the context of a computed tomography (CT)-based screening trial of high risk individuals. **The clinical question** to be tested after pre-validation relates to whether a serum/plasma biomarker has added diagnostic value to current tests (CT scan and/or PET scan) for the diagnostic evaluation of CT-detected pulmonary nodules. The number of samples available from contributing sites was sufficient to assemble a rapid pre-validation set from plasma.

3. *Controls*: The controls include blood samples from groups of individuals with matched age, sex, race, smoking status and smoking pack year history of smoking to the lung cancer cases. In addition, these controls include samples from subjects with non-malignant lung disease and malignant disease from other organ sites.

The biomarker context

1. *Rapid single biomarker Pre-validation Reference Sets*: A biomarker with demonstrated good performance in a discovery sample set, typically from one institution, needs to be tested in samples from *independent clinical populations* before going forward to validation in the combined pre-validation set or in a multi-center validation study.

2. Panel of biomarkers Pre-validation Reference Sets: The performance of an individual biomarker is insufficient alone but may have utility when *combined in a panel* with other biomarkers measured in the same sample set. In this evaluation, investigators will establish (in the “training set”) a combination marker decision rules. In the second set of specimens (the “test” set) they will evaluate the constructed rule in an independent sample of specimens to determine the rule’s operating characteristics (e.g., sensitivity, specificity, positive and negative predictive value). Since all researchers utilizing the combined pre-validation set will be evaluating their markers on essentially identical sets of specimens and contributing the data from their assays to the central database of assay results, over time the reference set will become progressively more valuable as the number of possible marker combinations that may be constructed and tested using this reference set expand.

3. Phase II biomarker Validation Reference Set: For single or combined biomarkers who have passed **1.** or **2.**

Sample collection and standardization

A critical aspect of serum biomarker validation is to be able to confirm the predictive value of biomarkers beyond the heterogeneity in sample collection, preparation, storage and shipping. The focus initially is on obtaining retrospective samples from institutions where cases and controls were collected, processed, and stored under the same protocol. **See Appendix D.**

Review process

All applications will be reviewed by the Specimen Sharing Committee and the review will be based on the scientific merit of the application. After providing specific details related to the sample set(s) being requested and institutional approval to use these sets, the investigator requesting access is then expected to address the following topics as provided on the application form in relation to his/her biomarker and future intentions. The application is expected to contain at least preliminary analysis of lung cancer samples.

Clinical Relationship

Background and Significance

Preliminary Data & Methods

Data Analysis Plan

Collaboration

Future Plans

Receipt and Review Schedule:

Letter of Intent due date: July 1 and January 1

First Application Receipt Date: August 1 and February 1

Specimen Sharing Committee Review Dates: September 2007 and March 2008

In essence, the Specimen Sharing Committee will review the applications prior to granting access to Reference Set samples. These criteria are established by the LCBG before the Reference Set samples become available. For each review conducted, it is expected that an adequate biostatistical critique will be provided by involvement of the EDRN Data Management and Coordinating Center (DMCC) or SPORE statistical group to ensure that appropriate consideration is given to statistical concerns of the proposal.

Upon receiving an inquiry or request regarding access to the Reference Set(s) samples the NCI Program staff committee member will be notified to send an application form and any other relevant documents to the investigator. After the completed application has been returned, the NCI Program staff committee member will then forward it to the members of the Specimen Sharing Committee. The Committee, in a timely manner (within one month), will review and discuss the application and offer a recommendation of whether 1) the investigator should be sent the requested Reference Set(s) samples, 2) further clarification or revision are needed, or 3) the request is considered low priority and deferred.

- 1) If approval is given, the LCBG will be notified at its next meeting (or by email if extenuating circumstances arise) by the Specimen Sharing Committee Chair (or Co-chair). In principle, the LCBG will concur with all approvals recommended by Specimen Sharing Committee unless special issues are raised. NCI Program Staff will then notify the repository facility in Frederick to prepare the materials needed for sending the appropriate Reference Set(s) samples.
- 2) If the application is unfunded at the time the request is submitted and is approved, the LCBG will provide to the investigator a letter of commitment. This obligation of samples will last for one year from the approval date.
- 3) If further clarification is needed, the LCBG will inform the NCI Program staff committee member what concerns or questions remain with the application. The NCI Program staff committee member will then communicate with the investigator of these issues to ask for a resubmission.
- 4) If the request is deemed low priority and deferred, the LCBG will provide the rationale to the NCI Program staff committee member why the request was deferred. This staff member will then relay this decision and its reasons to the investigator requesting access.

Timeline

It is expected that the review of applications will take approximately one month following submission of a complete application. If the application is approved, the requested Reference Set(s) samples will be sent to the applicant within two weeks of the approval date.

Contacts:

Karl Krueger, Ph.D.
Program Director
Cancer Biomarkers Research Group
Division of Cancer Prevention
National Cancer Institute
6130 Executive Plaza North, Suite 3136
Rockville, MD 20852
Telephone: (301) 594-1044
Fax: (301) 402-8990

Email: kruegerk@mail.nih.gov

Peter Ujhazy, M.D., Ph.D.
Program Director
Organ Systems Branch
OCTR, ODDES, NCI
6116 Executive Blvd. Room 7015
Rockville, MD 20852
Telephone: (301) 496-8528
Fax: (301) 402-5319
Email: pu5s@nih.gov
<http://spores.nci.nih.gov>

Appendix D. Specimen collection recommendations

NCI/EDRN/SPORE Lung Cancer Biomarkers Group

Adapted from NCI/FDA Proteomics Program Protocol

Standard Operating Procedure

Collection of Serum and Plasma Samples for Proteomic Analysis

I. Principle

The collection of human blood samples for biomarker analysis requires that patient sample collection, storage, transport and handling remain consistent within rigid guidelines for optimal results.

II. Materials

1. Requisition
2. Gloves
3. Sharps Container
4. Vacutainer needles, 20-22g and vacutainer hub or Butterfly needle, attached tubing and Luer adapter
5. Tourniquet
6. Antiseptic wipes
7. Red top glass, no additive, no clot activator with uncoated interior, vacutainer tubes (**BD 366430 for serum**) and **EDTA spray coated tube (BD 366643)**.
8. Bandages
9. Centrifuge
10. Polypropylene screw top freezer tubes, 2 ml.

III. Specimen

The specimen of choice for proteomic analysis at the NCI/FDA Proteomics Program is serum obtained from whole blood collected in red top vacutainer tubes with no additives or clot activators. The blood specimen should be allowed to clot for 40 - 50 minutes. The serum should be transferred to a transfer/storage tube **within 2 hours of collection**. Samples are then frozen at -80°C until testing is performed. The serum should be free of hemolysis and clots. Freezing and thawing cycles should be kept to a minimum. (Plasma samples can also be analyzed however every sample in the set must be plasma and the same anticoagulant used for all samples).

IV. Procedure

1. Identification of the patient is crucial. The person obtaining the blood specimen must ensure that the blood specimen being drawn is from the individual designated on the request form
2. Assemble the supplies to be used in obtaining the specimen. Label the tubes.
3. Put on disposable gloves.
4. The patient should be comfortably seated in a venipuncture chair. The arm should be positioned on a slanting armrest in a straight line from the shoulder to the wrist. The arm should not be bent at the elbow.
5. Apply a tourniquet 2 inches above the antecubital fossa or above area to be drawn with enough pressure to provide adequate vein visibility. Have the patient form a fist. Select the site for venipuncture.
6. Clean the forearm of the patient with antiseptic wipe in a circular motion beginning at the insertion site. Allow the antiseptic to dry.
7. Anchor the vein by placing the thumb 2 inches below the site and pulling the skin taut to prevent the vein from moving. The holding finger is placed below the site, not above, to prevent accidentally sticking the finger with the needle.
8. Using the dominant hand, insert either the vacutainer needle or the butterfly needle (if using vacutainer needle, attach hub first). Push the evacuated tube onto the vacutainer hub or the Luer adapter if using a butterfly.
9. Release the tourniquet once blood flow is established.
10. Carefully remove the tubes when full without dislodging the needle. The tube will automatically stop filling when the vacuum is gone leaving the tube approximately three-fourths full.
11. Lightly place a sterile gauze pad over the venipuncture site. Gently remove the needle.
12. Apply pressure to the site with sterile gauze. Apply bandage. Instruct the patient to leave the bandage on for at least 15 minutes.
13. Dispose of the needle in a sharps container.
14. Remove gloves and wash hands.

Plasma and buffy coat

1. Under the direction of a qualified and licensed physician, trained phlebotomists will collect blood from each donor into vacutainer that contain either no anticoagulant or an appropriate volume of anticoagulant K₂EDTA, to prevent clotting (**BD 366643**).
2. From each consenting donor, **10 mL** of whole blood will be collected for plasma collection.
3. The specimens are centrifuged immediately after blood draw at 1000 g (RCF) for 10 minutes at 4° C. The resultant plasma (assume 40% yield) is transferred into secondary centrifuge tubes.
4. Save the Buffy coat at -20°C for DNA extraction. No need to transfer buffy coat in another tube. DNA can be extracted from WBC after RBC lysis.
5. The secondary tubes are then centrifuged at 1500 g (RCF) at 4° C for 5 minutes to remove all potentially remaining cells.
6. Aliquots will be transferred into labeled cryovials and frozen at below -80° C within 2 hours of processing.

Serum

1. Approximately 10ml of blood is collected in a sterile vacutainer (**BD 366430**), red top no additive vacutainer)
2. Leave on the bench at room temperature for ~ 45 minutes to allow clot to form.
3. Spin at 1000g for 10 min in a refrigerated (4°C) centrifuge.
4. Transfer supernatant carefully into a polypropylene Eppendorf-style microfuge tube and store immediately at -80° C.

Dispose of all tubes and materials used to transfer patient samples in biohazardous waste.

V. Procedural notes

1. Do not draw from an IV, mastectomy or shunt arm.
2. Samples may also be drawn into a syringe and then dispensed into a vacutainer tube (before clotting) for processing.
3. **ORDER OF DRAW:** Blood collection tubes must be drawn in a specific order to avoid cross-contamination of additives between tubes. The recommended order of draw is:
First - non-additive tube (**BD 366430**)
Second –EDTA (**BD 366643**).

NOTE: Tubes with additives must be thoroughly mixed. Erroneous test results may be obtained when the blood is not thoroughly mixed with the additive.

Appendix E. Common Clinical and Sample Data Elements **(UPDATE)**

E.1. REQUIRED

Group Name: Lung Reference Set
CDEs required for one or more study groups

795 Proposed study group
Required

- | | |
|---|--|
| <input type="checkbox"/> 1 Set A Cases: Lung cancer (CXR) | <input type="checkbox"/> 2 Set A Cases: Lung cancer (CT) |
| <input type="checkbox"/> 3 Set A Controls: High Risk | <input type="checkbox"/> 4 Set A Controls: Suspicious lung lesions |
| <input type="checkbox"/> 5 Set A Controls: Other cancers | <input type="checkbox"/> 6 Set B Cases: Lung cancer (CT screening) |
| <input type="checkbox"/> 7 Set B Controls: High risk | <input type="checkbox"/> 8 Set B Controls: CT nodule |
| <input type="checkbox"/> 9 Set B Controls: No nodule | <input type="checkbox"/> 10 Set B Controls: Other cancers |

422 EDRN Site ID 
No mapping necessary, auto assigned by DMCC

1063 Site Participant ID
Required

421 EDRN Participant ID 
No mapping necessary, auto assigned by DMCC

796 Date participant signed consent form 
Required

434 Gender (What is your gender?)
Required

- | | |
|--|-----------------------------------|
| <input type="checkbox"/> 1 Male | <input type="checkbox"/> 2 Female |
| <input type="checkbox"/> 9 Unknown/refused | |

436 Race (What is your race? Check all that apply.)
Required

- | | |
|--|--|
| <input type="checkbox"/> 1 White | <input type="checkbox"/> 2 Black or African-American |
| <input type="checkbox"/> 3 American Indian or Alaska Native | <input type="checkbox"/> 4 Asian |
| <input type="checkbox"/> 7 Native Hawaiian or other Pacific Islander | <input type="checkbox"/> 97 Other, specify: |
| <input type="checkbox"/> 99 Unknown/refused | |

793 Race (Other,specify)
Required

441 Ever smoke cigarettes regularly, at least one a day for a year or more? (Did you ever smoke cigarettes regularly, at least one a day for a year or more?)
Required

No
0

Unknown/refused
9

Yes
1

443 Currently smoke at least one cigarette a day? (Do you currently smoke cigarettes regularly, at least one a day?)
Required

No
0

Unknown/refused
9

Yes
1

1213 Average number of packs smoked per day?
Required

444 Age quit smoking cigarettes? (How old were you when you permanently quit smoking cigarettes?)
Required

1272 Living status:
Required

Alive with disease
1

Dead
3

Alive with no evidence of disease (NED)
2

1218 Last date known alive:
Required

1048 Date of death
Required

792 Ever had cancer [other than basal/squamous cell skin cancer] confirmed by a doctor? (Have you ever had cancer [other than basal/squamous cell skin cancer] confirmed by a doctor?)
Required

No
0

Unknown/refused
9

Yes
1

451 Histologic confirmation?
Required

No
0

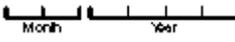
Yes
1

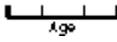
447 Cancer type/location

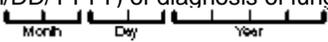
Required

- | | |
|--|---|
| <input type="checkbox"/> 1 Bladder | <input type="checkbox"/> 2 Bone |
| <input type="checkbox"/> 3 Brain | <input type="checkbox"/> 4 Breast |
| <input type="checkbox"/> 5 Cervix | <input type="checkbox"/> 6 Colon |
| <input type="checkbox"/> 7 Esophagus | <input type="checkbox"/> 8 Head & neck (mouth, nose, and throat) |
| <input type="checkbox"/> 9 Kidney | <input type="checkbox"/> 10 Liver |
| <input type="checkbox"/> 11 Leukemia | <input type="checkbox"/> 12 Lung |
| <input type="checkbox"/> 13 Lymphoma, including Hodgkins | <input type="checkbox"/> 14 Ovary |
| <input type="checkbox"/> 15 Pancreas | <input type="checkbox"/> 16 Prostate |
| <input type="checkbox"/> 17 Rectum | <input type="checkbox"/> 18 Skin (melanoma, no basal or squamous) |
| <input type="checkbox"/> 19 Stomach | <input type="checkbox"/> 20 Thyroid |
| <input type="checkbox"/> 21 Uterus | <input type="checkbox"/> 24 Testis |
| <input type="checkbox"/> 26 Vagina | <input type="checkbox"/> 97 Other, specify: |
| <input type="checkbox"/> 99 Unknown/refused | <input type="checkbox"/> 999 Unknown/refused |

727 Cancer type/location (Other, specify)
Required

510 Date of diagnosis (MM/YYYY) 
Required

574 Age at diagnosis 
Required

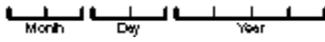
1216 Date (MM/DD/YYYY) of diagnosis of lung cancer (histological or cytopathological report) 
Required

1217 Date of thoracotomy (MM/DD/YYYY):
Required for cases, sets A and B

1234 Chest X-ray date: 
Desired for Set A

1235 Chest X-ray nodule size (cm): 
Desired for Set A

1236 Chest X-ray nodule location:
Desired for Set A

1237 Chest CT date: 
Desired for Set A

1238 Chest CT nodule size (cm): 
Desired for Set A

1239 Chest CT nodule location:

Desired for Set A

1240 Reported adenopathy > 1 cm in the mediastinum at time of diagnosis?
Desired for Set A

786 Lung T-Stage, Pathologic
*Required**

**If Pathologic stage is not available, provide Clinical stage.*

- | | | | |
|--------------------------|-----|--------------------------|------|
| <input type="checkbox"/> | pT0 | <input type="checkbox"/> | pTis |
| 2 | | 3 | |
| <input type="checkbox"/> | pT1 | <input type="checkbox"/> | pT2 |
| 4 | | 5 | |
| <input type="checkbox"/> | pT3 | <input type="checkbox"/> | pT4 |
| 6 | | 7 | |
| <input type="checkbox"/> | pTX | | |
| 8 | | | |

789 Lung N-Stage, Pathologic
*Required**

**If Pathologic stage is not available, provide Clinical stage.*

- | | | | |
|--------------------------|-----|--------------------------|-----|
| <input type="checkbox"/> | pNX | <input type="checkbox"/> | pN0 |
| 1 | | 2 | |
| <input type="checkbox"/> | pN1 | <input type="checkbox"/> | pN2 |
| 3 | | 4 | |
| <input type="checkbox"/> | pN3 | | |
| 5 | | | |

790 Lung M-Stage, Pathologic
*Required**

**If Pathologic stage is not available, provide Clinical stage.*

- | | | | |
|--------------------------|-----|--------------------------|-----|
| <input type="checkbox"/> | pMX | <input type="checkbox"/> | pM0 |
| 1 | | 2 | |
| <input type="checkbox"/> | pM1 | | |
| 3 | | | |

785 Lung T-Stage, Clinical
*Required**

**If Pathologic stage is not available, provide Clinical stage.*

- | | | | |
|--------------------------|----|--------------------------|-----|
| <input type="checkbox"/> | T0 | <input type="checkbox"/> | Tis |
| 2 | | 3 | |
| <input type="checkbox"/> | T1 | <input type="checkbox"/> | T2 |
| 4 | | 5 | |
| <input type="checkbox"/> | T3 | <input type="checkbox"/> | T4 |
| 6 | | 7 | |
| <input type="checkbox"/> | TX | | |
| 8 | | | |

787 Lung N-Stage, Clinical
*Required**

**If Pathologic stage is not available, provide Clinical stage.*

- | | | | |
|--------------------------|----|--------------------------|----|
| <input type="checkbox"/> | NX | <input type="checkbox"/> | N0 |
| 1 | | 2 | |
| <input type="checkbox"/> | N1 | <input type="checkbox"/> | N2 |
| 3 | | 4 | |
| <input type="checkbox"/> | N3 | | |
| 5 | | | |

788 Lung M-Stage, Clinical
*Required**

**If Pathologic stage is not available, provide Clinical stage.*

- 1 MX
- 3 M1

- 2 MO

1257 Histologic type:
Required

- | | |
|---|--|
| <input type="checkbox"/> 1 Squamous cell carcinoma | <input type="checkbox"/> 2 Papillary squamous cell carcinoma |
| <input type="checkbox"/> 3 Clear cell squamous cell carcinoma | <input type="checkbox"/> 4 Small cell squamous cell carcinoma |
| <input type="checkbox"/> 5 Basaloid squamous cell carcinoma | <input type="checkbox"/> 6 Small cell carcinoma |
| <input type="checkbox"/> 7 Combined small cell carcinoma | <input type="checkbox"/> 8 Adenocarcinoma |
| <input type="checkbox"/> 9 Acinar adenocarcinoma | <input type="checkbox"/> 10 Papillary adenocarcinoma |
| <input type="checkbox"/> 11 Bronchioloalveolar carcinoma | <input type="checkbox"/> 12 Non-mucinous |
| <input type="checkbox"/> 13 Mucinous | <input type="checkbox"/> 14 Mixed mucinous and non-mucinous or indeterminate |
| <input type="checkbox"/> 15 Solid adenocarcinoma with mucin formation | <input type="checkbox"/> 16 Adenocarcinoma with mixed subtypes |
| <input type="checkbox"/> 17 Large cell carcinoma | <input type="checkbox"/> 95 Other |
| <input type="checkbox"/> 99 Unknown/refused | |

504 Date of follow-up data collection 

905 New primary cancer [other than basal/squamous cell skin cancer] confirmed by a doctor since last routine study contact? (Have you been diagnosed with a new primary cancer [other than basal/squamous cell skin cancer] since your last routine study contact?)

Required

- | | |
|--|--------------------------------|
| <input type="checkbox"/> 0 No | <input type="checkbox"/> 1 Yes |
| <input type="checkbox"/> 9 Unknown/refused | |

742 Cancer type/location
Required

- | | |
|--|--|
| <input type="checkbox"/> 1 Bladder | <input type="checkbox"/> 2 Bone |
| <input type="checkbox"/> 3 Brain | <input type="checkbox"/> 4 Breast |
| <input type="checkbox"/> 5 Cervix | <input type="checkbox"/> 6 Colon |
| <input type="checkbox"/> 7 Esophagus | <input type="checkbox"/> 8 Head & neck (mouth, nose, and throat) |
| <input type="checkbox"/> 9 Kidney | <input type="checkbox"/> 10 Liver |
| <input type="checkbox"/> 11 Leukemia | <input type="checkbox"/> 12 Lung |
| <input type="checkbox"/> 13 Lymphoma, including Hodgkins | <input type="checkbox"/> 14 Ovary |

15 Pancreas

17 Rectum

19 Stomach

21 Uterus

26 Vagina

97 Other, specify:

16 Prostate

18 Skin (melanoma, no basal or squamous)

20 Thyroid

24 Testis

44 None

999 Unknown/refused

728 Cancer type/location (Other, specify)
Desired

513 Histologic confirmation?
Required

0 No

1 Yes

578 Age at specimen collection
Required

1064 Site Specimen ID
Required

533 Final storage
Required

Storage temperature at original site

1 Liquid nitrogen

4 -20°

6 Room temperature

3 -70°/-80°

5 4°

9 Unknown/refused

529 Specimen stored (type)
Required

2 Serum

25 Mononuclear cells

3 Plasma

95 Other

569 Date of specimen collection

Required (DMCC states this will be in Specimen Tracking System)

747 Additive used in blood collection?
Required

1 EDTA

3 Citrate

99 Unknown/refused

2 Heparin

4 None

751 Original blood sample was collected as:

Required

Fasting
1

Random
2

Unknown/refused
9

1248 Was sample collected and processed according to Standard Operating Procedures (SOPs)?

Required

No
0

Yes
1

Unknown/refused
9

530 Approximate total amount stored
Required

531 Approximate total amount stored (Unit)
Required

Microliters (mcl)
1

Milliliters (ml)
2

Liters (l)
6

945 Number of freeze-thaws:
Required

1267 Number of aliquots sent to NCI Frederick:
Required

E.2. DESIRED

**Group Name: Lung Reference Set
CDEs Desired**

799 Height [in inches] (What is your total current height in inches?)
Desired

1212 Weight (lbs) at time of diagnosis:
Desired

435 Hispanic or Latino (Are you Hispanic or Latino?)
Desired

No Yes
0 1
 Unknown/refused
9

442 Age first began smoking cigarettes regularly, at least one a day? (How old were you when you began smoking cigarettes regularly, at least one a day?) Age
Desired
Enter 999 if Unknown/Refused

445 Average number of cigarettes smoked per day? (During the time you have smoked, on average, how many cigarettes did you smoke per day?) Cigarettes
Desired
Enter 999 if Unknown/Refused

752 Ever smoke cigars regularly, at least one cigar a day, for a year or more? (Have you ever smoked cigars regularly, at least one a day, for a year or more?)
Desired

No Yes
0 1
 Unknown/refused
9

1211 Do you now or did you ever smoke a pipe for a year or longer?
Desired

No Yes
0 1
 Unknown/refused
9

1225 Do you now or did you ever chew tobacco for a year or longer?
Desired

No Yes
0 1
 Unknown/refused
9

1231 Is there a smoker in participant's household?
Desired

No Yes
0 1
 Unknown/refused
9

[1232](#) How many years was participant exposed to second hand smoke in the home? 
Desired

Occupational History

[1229](#) Has participant been exposed to any of the following known lung carcinogens greater than 8 hours per week for one year? (Check all that apply.)
Desired

- | | |
|--------------------------------------|-----------------------------------|
| <input type="checkbox"/> 1 Asbestos | <input type="checkbox"/> 2 Radon |
| <input type="checkbox"/> 3 Uranium | <input type="checkbox"/> 4 Silica |
| <input type="checkbox"/> 5 Coal dust | <input type="checkbox"/> 44 None |

[1230](#) Exposure to known lung carcinogens (other, specify):
Desired

Medication Use

[1233](#) If you use any illicit drugs, please specify:
Desired

Demographics

[1214](#) What is your living environment?
Desired

- | | |
|---|---|
| <input type="checkbox"/> 1 Live in own home | <input type="checkbox"/> 2 Live in assisted living |
| <input type="checkbox"/> 3 Live in a nursing home | <input type="checkbox"/> 4 Live with child/children |
| <input type="checkbox"/> 5 Live with friends | <input type="checkbox"/> 7 Other, specify: |

[1215](#) What is your living environment (other, specify):
Desired

[1226](#) If you live at home, who else lives with you?
Desired

[1227](#) If you have children, do they live within an hour's drive?
Desired

Alcohol Consumption

[997](#) Has participant ever had at least one drink of alcohol [beer, liquor, wine, or wine coolers] per month during a twelve-month period? (Have you ever had at least one drink of alcohol [beer, liquor, wine, or wine coolers] per month during a twelve-month period?)
Desired

- | | |
|--|--------------------------------|
| <input type="checkbox"/> 0 No | <input type="checkbox"/> 1 Yes |
| <input type="checkbox"/> 9 Unknown/refused | |

[782](#) On average, how many shots of hard liquor or mixed drinks do you drink? Count one shot (1 1/2 ounces) or one mixed drink as one drink.
Desired

- | | |
|---|--|
| <input type="checkbox"/> 4 None | <input type="checkbox"/> 5 <1 per week |
| <input type="checkbox"/> 6 1-6 per week | <input type="checkbox"/> 7 1-2 per day |
| <input type="checkbox"/> 8 3-5 per day | <input type="checkbox"/> 9 6+ per day |

[783](#) On average, how many glasses of wine do you drink? Count a four-ounce glass of wine as one drink.

Desired

- | | |
|---|--|
| <input type="checkbox"/> 4 None | <input type="checkbox"/> 5 <1 per week |
| <input type="checkbox"/> 6 1-6 per week | <input type="checkbox"/> 7 1-2 per day |
| <input type="checkbox"/> 8 3-5 per day | <input type="checkbox"/> 9 6+ per day |

[784](#) On average, how many glasses/cans of beer do you drink? Count a twelve-ounce can as one beer.

Desired

- | | |
|---|--|
| <input type="checkbox"/> 4 None | <input type="checkbox"/> 5 <1 per week |
| <input type="checkbox"/> 6 1-6 per week | <input type="checkbox"/> 7 1-2 per day |
| <input type="checkbox"/> 8 3-5 per day | <input type="checkbox"/> 9 6+ per day |

Medical History

[1271](#) Comorbidities:

Desired

- | | |
|--|--|
| <input type="checkbox"/> 1 Myocardial infarction | <input type="checkbox"/> 2 Congestive heart failure |
| <input type="checkbox"/> 3 Peripheral vascular disease | <input type="checkbox"/> 4 Cerebrovascular disease |
| <input type="checkbox"/> 5 Dementia | <input type="checkbox"/> 6 Chronic pulmonary disease |
| <input type="checkbox"/> 7 Connective tissue disease | <input type="checkbox"/> 8 Ulcer disease |
| <input type="checkbox"/> 9 Mild liver disease | <input type="checkbox"/> 10 Diabetes |
| <input type="checkbox"/> 11 Hemiplegia | <input type="checkbox"/> 12 Moderate or severe renal disease |
| <input type="checkbox"/> 13 Diabetes with end organ damage | <input type="checkbox"/> 14 Moderate or severe liver disease |
| <input type="checkbox"/> 15 Metastatic solid tumor | <input type="checkbox"/> 16 AIDS |

[1047](#) Cause of death:

Desired

[1266](#) Other lung diseases participant has:

Desired

- | | |
|---|--|
| <input type="checkbox"/> 1 COPD | <input type="checkbox"/> 2 Asthma |
| <input type="checkbox"/> 3 Pulmonary fibrosis | <input type="checkbox"/> 4 Sarcoidosis |

- 5 Granulomatosis
- 6 Cavitary lesion
- 7 Bronchiectasis

[1234](#) Chest X-ray date:
Desired for Set A

[1235](#) Chest X-ray nodule size (cm):
Desired for Set A

[1236](#) Chest X-ray nodule location:
Desired for Set A

[1237](#) Chest CT date:
Desired for Set A

[1238](#) Chest CT nodule size (cm):
Desired for Set A

[1239](#) Chest CT nodule location:
Desired for Set A

[1240](#) Reported adenopathy > 1 cm in the mediastinum at time of diagnosis?
Desired for Set A

- 0 No
- 1 Yes
- 9 Unknown/refused

[1268](#) PET scan date:
Desired

[1269](#) PET scan standardized uptake value (SUV) for the lesion:
Desired

- 1 Less than 2.5
- 2 Greater than or equal to 2.5

[1273](#) PET scan standardized uptake value (SUV) for the liver:
Desired

[1274](#) PET scan other hotspots:
Desired

- 1 Mediastinum
- 2 Bone
- 3 Brain
- 5 Adrenal
- 10 Liver
- 12 Lung
- 44 None

Lung

[1249](#) Pulmonary function test date (MM/DD/YYYY):
Desired

[1250](#) FVC liters
Desired

[1251](#) FVC % predicted:
Desired

- [1252](#) FEV1 liters:
Desired
 - [1253](#) FEV1 % predicted:
Desired
 - [1254](#) FEV1/FVC
Desired
 - [1255](#) DLCO liters/minute/mmhg
Desired
 - [1256](#) DLCO % predicted:
Desired
 - [1241](#) Histologic dimensions - height (cm): 
Desired
 - [1242](#) Histologic dimensions - width (cm): 
Desired
 - [1243](#) Histologic dimensions - depth (cm): 
Desired
 - [1244](#) Histologic dimensions - volume (ml): 
Desired
 - [1245](#) Distance/pleural margin (mm): 
Desired
 - [1246](#) Distance/bronchial margin (mm): 
Desired
 - [720](#) Anatomical site
Desired
- | | |
|---|---|
| <ul style="list-style-type: none"> <input type="checkbox"/> 1 Bladder <input type="checkbox"/> 3 Bowel peritoneum/serosa <input type="checkbox"/> 5 Cervix <input type="checkbox"/> 7 Diaphragm <input type="checkbox"/> 9 Omentum <input type="checkbox"/> 11 Pelvic wall <input type="checkbox"/> 13 Vagina <input type="checkbox"/> 15 Mainstem bronchus <input type="checkbox"/> 17 Upper lobe (UL) <input type="checkbox"/> 19 Middle lobe (ML) <input type="checkbox"/> 21 Renal pelvis <input type="checkbox"/> 25 Lymph node <input type="checkbox"/> 97 Other, specify: | <ul style="list-style-type: none"> <input type="checkbox"/> 2 Bladder peritoneum <input type="checkbox"/> 4 Corpus <input type="checkbox"/> 6 Cul-de-sac <input type="checkbox"/> 8 Fallopian tube <input type="checkbox"/> 10 Paracolic gutter <input type="checkbox"/> 12 Peritoneum/uterine serosa <input type="checkbox"/> 14 Ovary <input type="checkbox"/> 16 Carina <input type="checkbox"/> 18 Lower lobe (LL) <input type="checkbox"/> 20 Ureter <input type="checkbox"/> 22 Endometrium <input type="checkbox"/> 95 Other |
|---|---|

Cancer History

1210 Primary cancer treatment(s) received:

Desired

- | | |
|---|--|
| <input type="checkbox"/> 1 Chemotherapy | <input type="checkbox"/> 2 Radiation therapy |
| <input type="checkbox"/> 3 Surgery | <input type="checkbox"/> 9 Unknown/refused |
| <input type="checkbox"/> 95 Other | |

1258 Chemotherapy treatment(s) received: [specify drug(s)]

Desired

1259 Chemotherapy start date: (MM/YYYY)

Month Day Year

Desired

1260 Chemotherapy end date: (MM/YYYY)

Month Day Year

Desired

1261 Radiation therapy received: (specify site)

Desired

1262 Radiation therapy start date: (MM/YYYY)

Month Year

Desired

1263 Radiation therapy end date: (MM/YYYY)

Month Year

Desired

1264 Total dose to chest: (cGy)

_____ . _____

Desired

1265 Total dose to other organ(s): (cGy)

_____ . _____

Desired

1282 Radiation therapy (RT) volume [cubic mm]:

Desired

1281 Radiation therapy (RT) complications:

Desired

- | | |
|---|--|
| <input type="checkbox"/> 1 Pneumonitis requiring steroids | <input type="checkbox"/> 2 Esophagitis requiring IVF |
| <input type="checkbox"/> 3 Feeding tube | <input type="checkbox"/> 4 Hospitalization |
| <input type="checkbox"/> 97 Other, specify: | |

1279 Response to treatment (imaging criteria)

Desired

- | | |
|-------------------------------|--------------------------------|
| <input type="checkbox"/> 0 No | <input type="checkbox"/> 1 Yes |
|-------------------------------|--------------------------------|

1278 Date of response (MM/DD/YYYY)

Month Day Year

Desired

1220 Disease status:

Desired

- | | |
|---|---|
| <input type="checkbox"/> 3 Stable Disease | <input type="checkbox"/> 4 Progression |
| <input type="checkbox"/> 5 Recurrence/relapse | <input type="checkbox"/> 6 No evidence of disease |

Unknown/refused
9

1276 Disease free survival (DFS)?
Desired

No
0

Yes
1

1280 Overall survival (OS) in days:
Desired

1221 Recurrence date (MM/YYYY):
Desired

1222 Recurrence site:
Desired

Bladder
1

Brain
3

Cervix
5

Esophagus
7

Kidney
9

Leukemia
11

Lymphoma, including Hodgkins
13

Pancreas
15

Rectum
17

Stomach
19

Uterus
21

Vagina
26

Other
95

Bone
2

Breast
4

Colon
6

Head & neck (mouth, nose, and throat)
8

Liver
10

Lung
12

Ovary
14

Prostate
16

Skin (melanoma, no basal or squamous)
18

Thyroid
20

Testis
24

None
44

Unknown/refused
99

1277 Time to local recurrence (TTLR):
Desired

1223 Progression date (MM/YYYY)
Desired

1275 Time to progression in days (TTP):
Desired

1224 Progression site:
Desired

Bladder
1

Brain
3

Cervix

Bone
2

Breast
4

Colon

- | | |
|---|--|
| 5 <input type="checkbox"/> Esophagus | 6 <input type="checkbox"/> Head & neck (mouth, nose, and throat) |
| 7 <input type="checkbox"/> Kidney | 8 <input type="checkbox"/> Liver |
| 9 <input type="checkbox"/> Leukemia | 10 <input type="checkbox"/> Lung |
| 11 <input type="checkbox"/> Lymphoma, including Hodgkins | 12 <input type="checkbox"/> Ovary |
| 13 <input type="checkbox"/> Pancreas | 14 <input type="checkbox"/> Prostate |
| 15 <input type="checkbox"/> Rectum | 16 <input type="checkbox"/> Skin (melanoma, no basal or squamous) |
| 17 <input type="checkbox"/> Stomach | 18 <input type="checkbox"/> Thyroid |
| 19 <input type="checkbox"/> Uterus | 20 <input type="checkbox"/> Testis |
| 21 <input type="checkbox"/> Vagina | 24 <input type="checkbox"/> None |
| 26 <input type="checkbox"/> Other | 44 <input type="checkbox"/> Unknown/refused |
| 95 | 99 |

Medical History

1045 Performance status (Which of the following options would you say describes your current performance status?)

Desired

- | | |
|---|--|
| <input type="checkbox"/> Fully active, able to carry on all pre-disease performance without restriction | <input type="checkbox"/> Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work |
| <input type="checkbox"/> Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours | <input type="checkbox"/> Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours |
| <input type="checkbox"/> Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair | |

1247 Time from blood draw to first freeze: (value)

Desired



Appendix F. Material Transfer Agreement form**Agreement for the Transfer of Materials to the NCI**

This Agreement is used for transfers of human biospecimens in research studies to determine the robustness of new cancer biomarkers for possible investigation in Phase II validation studies with the Lung Cancer Biomarkers Group represented by the Special Programs of Research Excellence (“SPORE”) and the Early Detection Research Network (“EDRN”), multi-institution networks of investigators funded by the National Cancer Institute (“NCI”).

PROVIDER: *[please insert name of institution and scientist providing MATERIAL to the NCI]*

1. The PROVIDER agrees to transfer to the NCI the following MATERIAL, which is the property of the PROVIDER: *[please insert description of samples to be provided]* (hereinafter referred to as “MATERIAL”).
2. THIS MATERIAL MAY NOT BE USED IN HUMAN SUBJECTS.
3. The MATERIAL has been collected from human subjects. The NCI will not receive any private identifiable patient information. The MATERIAL has been collected under an IRB approved protocol *[please insert title and number of the IRB approved protocol]*, which includes all necessary informed consents and authorizations which disclose potential redistributions of the MATERIAL in accordance with Section 5 of this Agreement, in accordance with all applicable federal regulations for the protection of human subjects, including, as applicable, 45 CFR Part 46, “Protection of Human Subjects,” and the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164, under Assurance Number *[please insert number]*. NCI will only transfer MATERIAL and any clinical data, results and raw data relating to the MATERIAL that is stripped of identifiable private information.
4. Any MATERIAL delivered pursuant to this Agreement is understood to be experimental in nature and may have hazardous properties. THE PROVIDER MAKES NO REPRESENTATIONS AND EXTENDS NO WARRANTIES OF ANY KIND, EITHER EXPRESSED OR IMPLIED. THERE ARE NO EXPRESS OR IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, OR THAT THE USE OF THE MATERIAL WILL NOT INFRINGE ANY PATENT, COPYRIGHT, TRADEMARK, OR OTHER PROPRIETARY RIGHTS. No indemnification for any loss, claim, damage, or liability is intended or provided by any party under this agreement. Unless prohibited by law, the NCI assumes all liability for claims for damages against it by third parties which may arise from the NCI’s use, storage or disposal of the MATERIAL except that, to the extent permitted by law, the PROVIDER shall be liable to the NCI when the damage is caused by the gross negligence or willful misconduct of the PROVIDER.
5. The MATERIAL will be redistributed by the NCI’s contractor on behalf of NCI in accordance with criteria established by the EDRN Executive Committee and the NCI Division of Cancer Prevention’s Cancer Biomarkers Research Group to qualified investigators and their institutions (RECIPIENTS) who shall first have executed written agreements with the NCI.

6. The MATERIAL is provided at no cost.

7. Any inventions arising from a RECIPIENT's use of the MATERIAL shall be governed by U.S. patent law. RECIPIENTS shall retain title to any patent or other intellectual property rights in inventions made by its employees in the course of conducting research with the MATERIAL. No right, title or interest in any such invention is transferred by virtue of this Agreement.

The PROVIDER and the NCI must sign both copies of this letter and return one signed copy to the PROVIDER. The PROVIDER will then send the MATERIAL.

FOR THE PROVIDER:

Provider Scientist: _____

Provider Organization: _____

Name of Authorized Official: _____

Title of Authorized Official: _____

Address: _____

Signature of Authorized Official: _____

Date: _____

FOR THE NCI:

Name of Authorized Official: _____

Title of Authorized Official: _____

Signature of Authorized Official: _____

Date: _____