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Current Readings: Blood-Based Biomarkers for Lung Cancer

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Introduction

Lung cancer is the leading cause of cancer death worldwide, primarily due to diagnosis of the disease at an advanced stage, when mortality is high. Surgical resection of early (stage Ia-Ib) lung cancer has a 10-year survival of 88% [1] or more. The recent published data from the National Lung Screening Trial (NLST) confirmed that early diagnosis of lung cancer by CT scan screening improves survival by 20% when compared to chest radiography [2]. However, screening CT scan has a high false-positive rate (25% in NLST and up to 50% in our series [3]) which leads to the need for additional CT scans with their attendant radiation exposure, PET scans, and/or invasive procedures such as bronchoscopy with biopsy or transthoracic fine needle aspiration with even greater risks, costs (up to 20-40-fold more than watchful waiting) and anxiety. Current research efforts focus on identifying sensitive and specific blood biomarkers for early detection of lung cancer. Studies on blood biomarkers suggest that circulating biomarkers have the best potential to be a cost-effective method for early lung cancer detection. We review recent advances in blood-based lung cancer biomarkers which have the potential to be clinically useful in the near future.

Unlocking Biomarker Discovery: Large Scale Application of Aptamer Proteomic Technology for Early Detection of Lung Cancer.

Ostroff RM, Bigbee WL, Franklin W, Gold L, Mehan M, Miller YE, Pass HI, Rom WN, Siegfried JM, Stewart A et al. *PloS one* 2010, **5**(12):e15003.

Ostroff et al. conducted a multi-center case control study to study the utility of aptamer-based assay as an early lung cancer biomarker in 1326 patients, of which 291 patients had NSCLC.

Lung cancer in its earliest stages may secrete or release proteins that can be identified and measured in the blood, and may give rise to auto-antibodies. Some of the proteins may be transient, whereas the auto-antibodies persist. In addition, the tumor-associated proteins are in small amounts and may be overwhelmed by common proteins like albumin. Measurement

of circulating proteins for early diagnosis, prognosis, and monitoring of therapy has long been a challenge due to the large number of proteins and their many dynamic variants. Private industry has entered this arena with their ability to capture the latest advances in technology, create high-throughput platforms, and amass a large funding base. One biotechnology company, SomaLogic, has developed a novel DNA-based aptamer to tackle the challenge of developing protein-based biomarkers [4]. Aptamers are a class of nucleic acid-based molecules, short single-stranded oligonucleotides that fold into intricate structures that bind with high affinity to different proteins, even at femtomolar concentrations. Using a multiplex proteomic assay against these aptamers, Ostroff et al. conducted a multi-center case-control study (n=1326) of NSCLC from four independent sites (291 cancer cases vs. 1035 smoker controls) [5]. In this study, the investigators measured 813 proteins using this new aptamer-based technology, and identified 44 candidate biomarkers. Of these, twelve highly discriminatory proteins (cadherin-1, CD30 ligand, endostatin, HSP90 α , LRIG3, MIP-4, pleiotrophin, PRKCI, RGM-C, SCF-sR, sL-selectin, and YES) were used to develop a panel of lung cancer markers. The investigators then tested this panel on a training set containing 213 cancer cases (62% stage I-II, 56% adenocarcinomas and 33% squamous cell carcinomas). Cancer-case aptamer levels were compared to levels in smoker controls (n=772), which included some patients with COPD and benign nodules. The investigators reported the ability to distinguish cancers from controls with 91% sensitivity, 84% specificity and an impressive AUC of 0.91. In the validation step, using 78 cases (with similar cancer case profile--63% stage I-II and 63% adenocarcinoma) vs. 263 controls, this panel was able to discriminate NSCLC from controls with 89% sensitivity and 83% specificity, and AUC of 0.90. The 12 identified proteins included 6 that were up-regulated and 6 that were down-regulated in the serum from lung cancer study subjects. These proteins are known to play roles in cell movement and growth, cell-cell adhesion, inflammation and immune monitoring.

Biomarkers to Help Guide Management of Patients With Pulmonary Nodules

Patz EF, Jr., Campa MJ, Gottlin EB, Trotter PR, Herndon JE, 2nd, Kafader D, Grant RP, Eisenberg M, American Journal of Respiratory and Critical Care Medicine 2013, **188**(4): 461-465

Patz et al. subjected serum data and nodule size to a logistic regression model to help guide management of 7-30 mm-sized indeterminate pulmonary nodules in a training set of 509 patients, of which 298 were lung cancer, with high sensitivity/specificity.

In another proteomic study, using known protein targets, Patz and colleagues reported that serum levels of carcinoembryonic antigen (CEA), α 1-antitrypsin (ATT), and squamous cell carcinoma (SCC) antigen have the potential to help guide management of 7-30 mm-sized indeterminate pulmonary nodules [6]. This study of 509 high-risk subjects with pulmonary nodules greater than 5 mm included 298 cancer cases (54% stage I-II and 49% adenocarcinoma) and 211 benign nodule controls. Using a logistic regression model accounting for four features, nodules size (small <1 cm, intermediate 1 to <3 cm, and large $\geq$ 3 cm), and levels of CEA, ATT, and SCC, the overall sensitivity, specificity, PPV and NPV were, 82%, 84%, 88%, and 76%, respectively. This biomarker assay was validated in a cohort of 203 cancer cases vs. 196 controls with similar results to the training set in sensitivity, specificity, PPV and NPV (80%, 89%, 89%, and 81%, respectively).

Variant Ciz1 is a Circulating Biomarker for Early-Stage Lung Cancer.

Higgins G, Roper KM, Watson IJ, Blackhall FH, Rom WN, Pass HI, Ainscough JF, Coverley D.: Proceedings of the National Academy of Sciences of the United States of America 2012, **109**(45):E3128-3135.

Higgins et al. first identified a variant protein, Ciz1, which was found to be elevated in lung cancer patients and then tested its utility as a biomarker in cohort of 119 lung cancer patients and 51 smoker controls.

Higgins et al. demonstrated that variant Ciz1 level on immunoblots is useful in detecting early stage lung cancer. *Ciz1*, a nuclear matrix-associated DNA replication factor, functions to promote initiation of DNA replication and coordinates the functions of cyclin E- and A-dependent protein kinases [7]. In this study, a stable Ciz1 variant, which lacks part of the c-terminal domain involved in nuclear matrix attachment, was found to be uniquely present only in tumor cells. Using an affinity-purified polyclonal antibody against peptide at exon14b/exon 15, variant Ciz1 was identified in plasma of lung cancer patients (n=119, 48% stage I-II NSCLC and 21% limited stage SCLC) by western blot, but not in plasma of healthy subjects (n=51). The presence of variant Ciz1 in the serum enabled the investigators to accurately distinguish cancer patients from controls (AUC=0.958). These results still need to be validated, and an ELISA assay must be developed if the marker is to be clinically useful.

Autoantibodies in Lung Cancer: Possibilities for Early Detection and Subsequent Cure.

Chapman CJ, Murray A, McElveen JE, Sahin U, Luxemburger U, Tureci O, Wiewrodt R, Barnes AC, Robertson JF: Thorax 2008, **63**(3):228-233.

Chapman et al. studied a set of autoantibodies as potential biomarkers and validated them in 3 groups of cohort that consist of patients with various stages of lung cancer.

The presence of lung malignant cells can activate the immune system and induce autoimmunity to autologous cellular antigens. Autoantibodies in response to tumor-associated antigens (TAAs) have been identified in human sera of cancer patients using high-throughput analysis. In a study by Chapman and colleagues, seven cancer-associated proteins (p53, c-myc, HER2, NY-ESO-1, CAGE, MUC1, and GBU4-5) were selected as markers of lung cancer [8]. Using an ELISA assay, the combination of these seven autoantibody assays had a panel sensitivity of 76% and specificity of 92%. The majority of the cancer cases had significantly elevated levels of autoantibody response compared to normal controls ($P<0.05$). In their validation trial, the autoantibodies selected included p53 (tumor suppressor gene), NY-ESO-1 and CAGE (cancer testis antigens), GRU4-5 (protein that encodes DEAD box domain), SOX2 (protein that induces autoantibody production in small-cell lung cancer), and Annexin I (phospholipid-binding protein) [9]. The validation trial included patients from three different centers. The subjects were divided into two groups: Group 1 (n=145) included current smokers, mostly >60 years of age, with Stage I-II NSCLC and control smokers; Group 2 (n=241) included stage III-IV lung cancer patients with unknown smoking status and control smokers. In group 1, only four antigens (p53, NY-ESO-1, CAGE, and GBU4-5) were measured, which yielded a sensitivity of 36% and specificity of 91%, with AUC of 0.71 (SE = 0.03). In group 2, all six antigens were measured, producing a sensitivity of 34% and specificity of 91%; AUC = 0.63 (SE = 0.03). The authors projected that using an at-risk population with lung cancer prevalence of 20 cases per 1000, this test would produce a negative predictive value (NPV) of 98.6%. They tested their hypothesis in a third group of patients that included a mix of stage I-IV NSCLC and smoker controls, and using the same six antigens, they achieved similar results in sensitivity (32%), specificity (91%), AUC (0.64), and NPV (98.6%). This panel of

autoantibodies is marketed as the *EarlyCDT®*-Lung test, and is the first clinically available biomarker test for the early detection of lung cancer. Studies in four additional cohorts of patients (n=574), including patients with both small cell lung cancer and NSCLC, subsequently confirmed these results [10].

Molecular Analysis of Plasma DNA for the Early Detection of Lung Cancer by Quantitative Methylation-Specific PCR.

Ostrow KL, Hoque MO, Loyo M, Brait M, Greenberg A, Siegfried JM, Grandis JR, Gaither Davis A, Bigbee WL, Rom W et al: *Clinical Cancer Research* 2010, **16**(13):3463-3472.

Ostrow et al. identified DNA methylation in tumor suppressor genes and studied if the frequency of these methylation can be used as biomarkers to distinguish individuals with benign solid nodules and GGOs from patients with lung cancer.

One of the most common molecular changes in human neoplasia is global hypomethylation, but interestingly, hypermethylation of DNA may occur in the promoter region of genes, resulting in the silencing of important tumor suppressor genes. DNA hypermethylation occurs when a methyl group is added to the cytosine ring to form methyl cytosine; frequently this precedes CpG dinucleotides (CpG island). Tumor suppressor genes often contain these CpG islands in their promoter region, and methylation of the promoter region is associated with gene silencing. Ostrow et al. studied the frequency of promoter methylation in five candidate tumor suppressor genes (RarB, NISCH, B4GALT1, KIF1a, and DCC) in the plasma of subjects with solid nodules or “ground glass opacities” (GGOs) on CT scan [11]. The training set included plasma from 24 cancer-free individuals and 13 patients with histologically confirmed lung cancer. The investigators reported differences in methylation status in controls vs. cancers, respectively, of RarB (4% vs. 38%), NISCH (8% vs. 36%), KIF1a (4% vs. 27%), and DCC (0% vs. 54%). These four tumor suppressor genes were then tested in a separate independent validation step. The validation cohort from the NYU Early Detection Research Network (EDRN) Lung Cancer Biomarker Center was made up of greater than 20 pack-year smokers, age over 50, and was divided into 3 groups: controls (n=80) had normal CT scans, “GGOs” (n=23) had sub-solid nodules or ground glass opacities, and lung cancers (n=70) had mostly early stage NSCLC (49/70 stage I). Using a cutoff value determined by ROC curves, methylation of RarB ($p=0.02$), NISCH ($p=0.037$), KIF1a ($p=0.0003$), and DCC ($p=0.0002$) were significantly different when comparing controls vs. GGO vs. cancers. When these 4 genes were used to create a combination panel of biomarkers, the panel differentiated cancer vs. non-cancer with a sensitivity of 73% and specificity of 71% (area under the curve, AUC= 0.643).

MicroRNA Signatures in Tissues and Plasma Predict Development and Prognosis of Computed Tomography Detected Lung Cancer.

Boeri M, Verri C, Conte D, Roz L, Modena P, Facchinetti F, Calabrò E, Croce CM, Pastorino U, Sozzi G: *Proceedings of the National Academy of Sciences* 2011, **108**(9): 3713-3718.

Boeri et al. found that blood microRNA expression levels in two large CT scan screening cohorts were useful in diagnosing early stage lung cancer as well as in prognosticating the disease.

MicroRNAs (miRNAs) are small ~22 nt noncoding RNAs that serve to down-regulate gene expression by suppression of translation or degradation of messenger RNA (mRNA) [12]. Currently more than 1000 human miRNAs have been identified, which regulate over 60% of the human genome. Most are involved in cell proliferation, apoptosis, and differentiation.

Each miRNA functions to target hundreds of different mRNAs and genes, and multiple miRNAs can target an individual gene. MiRNAs are often deregulated in cancer. Previous studies in blood from prostate, colon, and skin cancer have shown remarkable sensitivity and specificity in differentiating cancers from controls. Boeri et al. explored the miRNA expression profiles of lung cancers, normal lung tissues, and plasma samples from cases and healthy controls [13]. Training set samples (n=19 cancer cases) were obtained from a CT scan screening cohort of 1,035 subjects. This cohort consisted of heavy smokers (median pack-year = 40), age 50-84 years old, and were 71% men. At the completion of the trial 38 subjects had been diagnosed with lung cancer (63% stage I and 71% with adenocarcinoma). Validation set samples (n=22 cancer cases) were obtained from the Multicentric Italian Lung Detection (MILD) CT scan screening trial of 2,352 current or former smokers, where 53 subjects had been diagnosed with lung cancer (53% stage Ia-Ib and 57% adenocarcinoma). Measurement of miRNA expression revealed up-regulation of miR-7 (1.3-fold), miR-21 (2.9-fold), miR-200b (1.3-fold), miR-210 (3-fold), miR-291-1 (1.6-fold), and miR-324 (1.3-fold); and down-regulation of miR-126 (0.4-fold), miR-451 (0.5-fold), miR-30a (0.6-fold), and miR-486 (0.5-fold), in tumors compared with normal lung. In the same study, using high-throughput Taqman microfluidic card type A (Applied Biosystems), the authors compared 40 cancer plasma samples (19 cancer cases collected 12-28 months before disease detection) to 81 benign controls. In this training set, a panel of 15 miRNAs was 90% sensitive and 80% specific for identifying those who developed lung cancer. In a subsequent validation set (n=15 cancer cases) the panel had 80% sensitivity and 90% specificity (AUC = 0.85, $P < 0.0001$) for identifying cancer prior to detection on CT scan. Also in this study, a panel of 9 different miRNAs was able to distinguish aggressive vs non-aggressive lung cancer with 80% sensitivity and 100% specificity in the validation set. Using plasma samples collected at time of surgery or cancer detection, the investigators also found a panel of 13 miRNAs was 84% sensitive and 80% specific in diagnosing lung cancer (n=19). This panel was further validated to discriminate cancer from controls with a sensitivity of 75% and a specificity of 100% (AUC = 0.88, $p < 0.0001$). Overall, in this extensive study, the investigators identified 21 plasma miRNAs which composed signatures of risk, diagnosis, and prognosis. These all belong to major pathways thought to be involved in lung carcinogenesis or tumor progression, supporting their possible utility as biomarkers for lung cancer. These pathways include: cellular aging (mir-19b, mir-17, mir-106), bronchoalveolar and hematopoietic stem cells' renewal (mir-486, mir-106a, 142-3p), tumor recurrence in stage I NSCLC (mir-27b; mir-106a; mir-19b; mir-15b mir-16, mi-21), and lung cancer aggressiveness (mir-221, mir-222).

Gene Expression Profiles in Peripheral Blood Mononuclear Cells Can Distinguish Patients with Non-Small Cell Lung Cancer From Patients with Nonmalignant Lung Disease.

Showe MK, Vachani A, Kossenkova AV, Yousef M, Nichols C, Nikonova EV, Chang C, Kucharczuk J, Tran B, Wakeam E et al.: Cancer research 2009, **69**(24):9202-9210.

Showe et al. identified a gene-expression panel that was sensitive and specific in lung cancer, and also found that their level was significantly decreased after surgical resection of the cancerous lesion.

It has been theorized that malignant cells could alter the gene expression of normal immune cells through a series of chemokine and cytokine interactions. Tumor specific gene-expression signatures of peripheral blood mononuclear cells (PBMC) have been identified in several non-hematopoietic cancers. Showe and colleagues identified a 29-gene signature in PBMCs that distinguished patients with non-small cell lung cancer (NSCLC) from non-cancer controls with similar risk factors [14]. The investigators collected PBMC from a series of histologically proven NSCLC patients and matched controls. After RNA extraction

and purification, human genome microarrays were performed to determine differential expression levels. Classification via a support vector machine with 10-fold cross validation identified a 29 gene-expression signature that accurately distinguished cancer from non-cancer controls. As anticipated, the genes included in the classifier were associated with specific immune function pathways including *CD28* and T-cell receptor signaling, calcium-induced T-cell apoptosis, and macrophage and monocyte phagocytosis. In an independent validation set, this classifier signature had 76% sensitivity and 82% specificity for distinguishing cancers from controls. In addition, 93% of cancer patients in this validation set showed a significant decrease in gene signature expression after surgical resection of the cancerous lesion. This NSCLC classifier performed more accurately with squamous cell histopathology and increased disease burden. In conjunction with radiographic screening, PBMC tumor-specific gene signatures can accurately distinguish NSCLC from controls with nonmalignant lesions. Comparative expression levels may be useful in detecting disease recurrence after resection.

Commentary

With the publication of U. S. Preventive Services Task Force draft Recommendation Statement recommending screening of lung cancer released in July, 2013, many predict an increase in health care resources used for the workup of indeterminate pulmonary nodules [15]. Most indeterminate nodules 4-8 mm are followed with sequential CT scans for watchful waiting while the larger ones (8-30 mm) are further evaluated using FDG-positron emission tomography (PET), bronchoscopy, CT-scan guided fine needle aspiration, or VATS surgery. Recent advances in the development of serum or plasma biomarkers as a non-invasive and cost-effective way to risk stratify patients for the presence of lung cancer will not only reduce the number of unnecessary invasive procedures, but may also lead to the earlier removal of malignant nodules and the avoidance of delays in diagnosis.

Over the last decade, research in this area has been increasing, and the impressive advances in technology have allowed for a broad range of biomarker development. A simple search on PubMed with key words “lung cancer” and “biomarker” produced more than 1,200 articles, including reports of markers in blood, sputum, airway epithelium, buccal cells, urine, and breath. A blood biomarker has the potential to be an ideal source due to its ease of acquisition. If a biomarker has been further studied to demonstrate a mechanistic property linking it to lung cancer, it may be more readily accepted for clinical use.

As noted, the most fruitful areas of research in blood-based biomarkers for lung cancer are: identification of proteins, protein panels or antibodies to tumor-associated antigens; analysis of epigenetic changes such as methylation; microRNA profiling; and gene expression profiling. Proteins, protein panels and auto-antibodies are an active area of research. As noted, the advances in high-throughput techniques, and the entry of private industry into this arena will speed the discovery and validation process. As described, Ostroff et al. had success using an aptamer-based assay. Patz, et al. used proteomic techniques to identify a small panel of protein markers which could be used to diagnose early lung cancer or distinguish benign from malignant nodules. Similarly, Integrated Diagnostics, a small biotechnology company developed an innovative system called selected reaction monitoring mass spectrometry (SRM-MS), to create a novel plasma base proteomic assay to discriminate malignant and benign nodules [16]. Vachani, et al., in this multicenter study which included a training set of 143 patients with stage IA NSCLC or benign lung nodules, a multivariate 13 protein panel was able to differentiate malignant and benign nodules with a sensitivity of 93% and specificity of 45%. In a validation set of 104 patients, the protein panel yielded a similar sensitivity of 90% and specificity of 27%, with PPV and NPV of 30% and 96% respectively (using an estimated cancer prevalence of 20%). Further analysis

by the investigator showed that these 13 proteins were mapped to 4 nuclear proteins (AHR, FOS, MYC, and NRF2) which have been linked to lung cancer and lung inflammation. Although the current focus of much research has been on proteomics and protein panels, individual proteins may still be useful as lung cancer biomarkers. As described, Higgins et al. showed that a single protein marker—variant *ciz1*—may be effective as well.

Auto-antibodies to tumor-associated antigens may persist in the circulating blood longer than the proteins themselves, and may be more easily detected. Chapman et al. identified a panel of auto-antibodies that is now a part of a clinically available test for the early detection of lung cancer. In another auto-antibody study by Rom et al., autoantibody biomarkers were tested in a cohort of high risk smokers (n=158) with screening CT scans [17]. This cohort included lung cancer patients (n=22), smokers with sub-solid nodules (n=46), smokers with benign solid nodules (n=55), and smokers with normal CT scans (n=25). A final panel of six TAAs (c-myc, Cyclin A, Cyclin B1, Cyclin D1, CDK2, and Survivin) had a sensitivity of 81%, specificity of 97% and AUC of 0.907 in discriminating cancer cases from smoker controls.

Research in miRNAs has shown their potential not only as biomarkers for diagnosis, but also as markers useful for classification, prognostication, and predicting or assessing therapeutic response. As noted, Boeri and colleagues identified 21 plasma miRNAs which composed signatures of risk, diagnosis, and prognosis. Other investigators have reported similarly promising results. Similarly, Huang and colleagues were able to distinguish small cell lung cancer and NSCLC using miRNA expression levels (miR-25 and miR-375; with a corresponding AUC 0.823 and 0.978 respectively) [18]. Using these two miRNAs, the authors were able to discriminate adenocarcinoma from squamous cell carcinoma with an accuracy of 96%. In patients with Stages I to IIIa NSCLC, the presence of four miRNAs helped predict overall survival (median survival times: miR-486, 30.3 months; miR-30d, 22.37 months; miR-1, 22.87 months; and miR-499, 23.73 months [19]. MiRNAs with their extensive involvement in the human transcription process and their molecular stability in human serum will be a major biomarker for future research.

Genetic and epigenetic changes have also been successfully investigated as lung cancer biomarkers. As described above, Ostrow et al. identified DNA methylation in tumor suppressor genes and showed that the frequency this methylation can be used as a biomarker to distinguish individuals with benign solid nodules and GGOs from patients with lung cancer. Looking at gene expression profiling in immune cells brings together the theory that the presence of malignancy activates the immune system and the idea that we can use genetic changes as biomarkers for lung cancer. Showe et al. identified a gene-expression panel in PBMCs that was both sensitive and specific for lung cancer.

Blood-based biomarkers have great potential for use in the near future; however, they also have their limitations. First, it is unclear if these markers are organ-specific or if they may identify other cancers as well. Studies have not shown if these tests can be reliably used in patients with additional medical conditions, e.g. autoimmune disorders, infections or inflammatory diseases. More research is also needed to validate blood biomarkers in patients who have had previous lung cancer treated with resection and/or chemotherapy. Additionally, these tests cannot yet be used as stand-alone tests, but must be integrated with CT scan imaging. McWilliams et al. showed that patient characteristics and pulmonary nodules' characteristics together can accurately predict probability of lung cancer [20]. In this study of two separate data bases (n=7,008 nodules and n=5,021 nodules), using models containing age, gender, family history, presence of emphysema, nodule size, nodule location, nodule type (solid vs. mixed), nodule count, and nodule shape, the authors were

able discriminate benign and malignant nodules with AUC more than 0.90. Adding a blood-based biomarker to this model may improve accuracy even further.

In summary, the goal in lung cancer management is to detect lung cancer at the earliest stage when prognosis is the most favorable. Screening for lung cancer with CT scan will surely improve early lung cancer detection; however, additional biomarkers are needed to differentiate benign nodules from malignant cancer. Not only do we want to be able to identify the benign nodules and prevent further radiation exposure, unnecessary workup, and patient anxiety, we need to avoid delays in diagnosing of early lung cancer and proceed with surgical resection or medical treatment.

References

1. The International Early Lung Cancer Action Program Investigators: Survival of Patients with Stage I Lung Cancer Detected on CT Screening. *New England Journal of Medicine*. 2006; 355(17):1–763-1771. [PubMed: 16822990]
2. Team* TNLSTR. Reduced Lung-Cancer Mortality with Low-Dose Computed Tomographic Screening. *New England Journal of Medicine*. 2011; 365(5):3–95-409. [PubMed: 21651388]
3. Greenberg AK, Lu F, Goldberg JD, Eylers E, Tsay J-C, Yie T-A, Naidich D, McGuinness G, Pass H, Tchou-Wong K-M, et al. CT Scan Screening for Lung Cancer: Risk Factors for Nodules and Malignancy in a High-Risk Urban Cohort. *PloS one*. 2012; 7(7):e–39403.
4. Gold L, Ayers D, Bertino J, Bock C, Bock A, Brody EN, Carter J, Dalby AB, Eaton BE, Fitzwater T, et al. Aptamer-based multiplexed proteomic technology for biomarker discovery. *PloS one*. 2010; 5(12):e–15004.
5. Ostroff RM, Bigbee WL, Franklin W, Gold L, Mehan M, Miller YE, Pass HI, Rom WN, Siegfried JM, Stewart A, et al. Unlocking biomarker discovery: large scale application of aptamer proteomic technology for early detection of lung cancer. *PloS one*. 2010; 5(12):e–15003.
6. Patz EF Jr. Campa MJ, Gottlin EB, Trotter PR, Herndon JE 2nd, Kafader D, Grant RP, Eisenberg M. Biomarkers to help guide management of patients with pulmonary nodules. *American journal of respiratory and critical care medicine*. 2013; 188(4):4–61-465. [PubMed: 23815714]
7. Higgins G, Roper KM, Watson IJ, Blackhall FH, Rom WN, Pass HI, Ainscough JF, Coverley D. Variant Ciz1 is a circulating biomarker for early-stage lung cancer. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109(45):E–3128-3135.
8. Chapman CJ, Murray A, McElveen JE, Sahin U, Luxemburger U, Tureci O, Wiewrodt R, Barnes AC, Robertson JF. Autoantibodies in lung cancer: possibilities for early detection and subsequent cure. *Thorax*. 2008; 63(3):2–28-233.
9. Boyle P, Chapman CJ, Holdenrieder S, Murray A, Robertson C, Wood WC, Maddison P, Healey G, Fairley GH, Barnes AC, et al. Clinical validation of an autoantibody test for lung cancer. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO*. 2011; 22(2):3–83-389.
10. Lam S, Boyle P, Healey GF, Maddison P, Peek L, Murray A, Chapman CJ, Allen J, Wood WC, Sewell HF, et al. EarlyCDT-Lung: an immunobiomarker test as an aid to early detection of lung cancer. *Cancer prevention research (Philadelphia, Pa)*. 2011; 4(7):1–126-1134.
11. Ostrow KL, Hoque MO, Loyo M, Brait M, Greenberg A, Siegfried JM, Grandis JR, Gaither Davis A, Bigbee WL, Rom W, et al. Molecular Analysis of Plasma DNA for the Early Detection of Lung Cancer by Quantitative Methylation-Specific PCR. *Clinical Cancer Research*. 2010; 16(13):3–463-3472.
12. Bartel DP. MicroRNAs: Genomics, Biogenesis, Mechanism, and Function. *Cell*. 2004; 116(2):2–81-297.
13. Boeri M, Verri C, Conte D, Roz L, Modena P, Facchinetti F, Calabrò E, Croce CM, Pastorino U, Sozzi G. MicroRNA signatures in tissues and plasma predict development and prognosis of computed tomography detected lung cancer. *Proceedings of the National Academy of Sciences*. 2011; 108(9):3–713-3718.

14. Showe MK, Vachani A, Kossenkov AV, Yousef M, Nichols C, Nikonova EV, Chang C, Kucharczuk J, Tran B, Wakeam E, et al. Gene expression profiles in peripheral blood mononuclear cells can distinguish patients with non-small cell lung cancer from patients with nonmalignant lung disease. *Cancer research*. 2009; 69(24):9–202-9210.
15. U.S. PSTF. Lung Cancer Screening, Topic Page. <http://www.uspreventiveservicestaskforce.org/uspstf/uspstflung.htm>
16. Vachani, A.; Pass, H.; Li, X.; Lam, S.; Chelsky, D.; Hood, L.; Kearney, P.; Fang, KC.; Massion, PP.; Rom, WN. D34 ADVANCES IN LUNG CANCER. Vol. A6122. American Thoracic Society; Philadelphia, PA: 2013. Development Of A Multi-Variate Plasma Proteomic Classifier For The Molecular Characterization Of Pulmonary Nodules..
17. Rom W, Goldberg J, Addrizzo-Harris D, Watson H, Khilkin M, Greenberg A, Naidich D, Crawford B, Eylers E, Liu D, et al. Identification of an autoantibody panel to separate lung cancer from smokers and nonsmokers. *BMC Cancer*. 2010; 10(1):2–34. [PubMed: 20047698]
18. Huang W, Hu J, Yang DW, Fan XT, Jin Y, Hou YY, Wang JP, Yuan YF, Tan YS, Zhu XZ, et al. Two microRNA panels to discriminate three subtypes of lung carcinoma in bronchial brushing specimens. *American journal of respiratory and critical care medicine*. 2012; 186(11):1–160-1167. [PubMed: 22753679]
19. Lu Y, Govindan R, Wang L, Liu PY, Goodgame B, Wen W, Sezhiyan A, Pfeifer J, Li YF, Hua X, et al. MicroRNA profiling and prediction of recurrence/relapse-free survival in stage I lung cancer. *Carcinogenesis*. 2012; 33(5):1–046-1054.
20. McWilliams A, Tammemagi MC, Mayo JR, Roberts H, Liu G, Soghrati K, Yasufuku K, Martel S, Laberge F, Gingras M, et al. Probability of cancer in pulmonary nodules detected on first screening CT. *The New England journal of medicine*. 2013; 369(10):9–10-919.