INTRODUCTION AND OBJECTIVES

1- Abstract

Several immune checkpoint inhibitors targeting the PD-1 pathway have been developed with clinical trials showing an approximately 20% durable response in unselected patients with advanced non-small cell lung cancer (NSCLC). At the moment, no clear biomarker exists to accurately predict anti-PD1/PDL1 tumor responsiveness. The goal of this study is to broadly discover and evaluate the utility of blood based biomarkers for use in measuring and predicting response to immunotherapy in patients with lung cancer.

2- Background

Despite significant advances in detection and therapy, lung cancer remains the number one cause of cancer related death in both men and women worldwide (1). Most patients are diagnosed at an advanced stage with poor overall survival. NSCLC is the most common histotype representing 85% of new diagnoses (2), and the overall five year survival rate of patients with NSCLC is 16% (*3*). Improved understanding of the interaction between tumors and host immune surveillance has enabled the development of several immune checkpoint inhibitors targeting the PD-1/PD-L1 pathway that have shown an approximate 20% durable response in unselected patients with advanced NSCLC, and improved response rates in select patients with PDL1 expression (Reck, NEJM 2016). Preliminary data suggest that detection of tumor PD-L1 protein expression on human cancers using chromogenic-based immunohistochemistry (IHC) in formalin-fixed paraffin embedded tissue samples (FFPE) may predict clinical response to PD-1/PD-L1 directed therapy (4,5). However, meaningful responses have also been observed in PD-L1 negative patients. Moreover, use of IHC may be limited by access to tissue as well as assay variability and interpretive subjectivity. In addition, conventional radiographic assessment of treatment response using WHO criteria (Response Evaluation Criteria in Solid Tumors [RECIST]) does not fully capture the response patterns of immunotherapeutic agents often necessitating frequent repeat imaging to assess a therapeutic response. Mutation burden and neoantigen expression have been evaluated as potential biomarkers for immunotherapy, however there is no validated biomarker to follow these changes yet. As a result, a biomarker that could accurately predict the patients most likely to derive benefit from immunotherapy, as well as monitor response to treatment would be of significant clinical utility.

One biomarker that is being increasingly utilized for the non-invasive monitoring of patients with various malignancies is cell-free DNA (cfDNA). cfDNA consists of short double-stranded DNA fragments shed into the blood by tumor cells undergoing apoptosis or necrosis. We and others have shown that cfDNA can be readily extracted and sequenced from NSCLC patient blood for mutation detection and disease monitoring (Thompson J et al. CCR 2016; Couraud S et al. CCR 2014; Oxnard GR et al. CCR 2014; Newman AM et al. Nature Medicine 2013). While surgically resected tumor or fine needle aspirate have traditionally been the source of tissue for molecular monitoring, these samples often provide insufficient and/or inferior quality DNA for sequencing, and can under-represent the full molecular heterogeneity of the patient's disease, especially in the setting of metastasis. cfDNA thus represents a non-invasive approach for patient monitoring and may allow for a "liquid biopsy" for genomic alterations of the primary or metastatic tumor, monitoring of the disease status, and/or development of resistance to therapy.

In this study, we will collect blood and urine specimens from patients receiving immunotherapy as part of their standard of care for the treatment of NSCLC. We will use various platforms for measurement of new biomarkers, and these biomarkers will be assessed for their ability to predict responsiveness

to immunotherapy, correlation with radiographic response, ability to detect lung cancer specific genetic changes present in the primary tumor (e.g analysis of cfDNA for EGFR mutations), and impact on prognosis (response to therapy, progression free survival and overall survival).

Portions of the blood and urine samples drawn will also be stored for future research.

3- Study Objectives
The overall objective is to develop a comprehensive system to collect blood and urine samples from patients with NSCLC receiving immunotherapy

Primary Outcome Variable
1. Discovery and validation of new blood based biomarkers that predict response to immunotherapy in NSCLC

Secondary Outcome Variable
1. Storage of samples for future research endeavors

4- Location
Abrahamson Cancer Center at the Hospital of the University of Pennsylvania and Penn Presbyterian Medical Center

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STUDY DESIGN

5- General Design

Single center, longitudinal cohort study of NSCLC patients receiving immunotherapy with biomarker evaluation.

This is an observational study. Biomarkers that are detected in CLIA certified laboratories and are already part of standard of care in the management of lung cancer patients (e.g. circulating tumor DNA) may be disclosed to the treating physician and care may be altered based on routine practice. Biomarkers that are in research development and/or not performed in CLIA certified laboratories will not be disclosed to treating physician and no treatment decisions will be based on these results.

The organizational structure will support the collection of peripheral blood and urine samples from subjects with NSCLC receiving immunotherapy for evaluation of circulating biomarkers. Physicians, nurse practitioners, and physician assistants will identify subjects with NSCLC who are scheduled to receive an immune checkpoint inhibitor as part of their routine clinical care at the University of Pennsylvania. The subjects will be consented for the collection of blood and urine samples. Given the exploratory nature of this study and that the hypotheses for circulating tumor material in NSCLC patients receiving immunotherapy will adapt as information from this analysis proceeds, the timeline and frequency of blood collection are not defined. The frequency of research blood collection will not exceed the lesser of 50 mL or 3 mL/kg in an 8 week period and will not occur more frequently than 2 times per week. Blood will generally be collected at the time of routine phlebotomy for the management of their disease, but in rare instances the

subject may be asked to provide only a research blood sample if the timing of the routine phlebotomy does not correlate with the timing necessary for the study. Urine specimens will be obtained in a sterile specimen cup.

Duration

The total study duration will be 5 years. Subjects will be considered active participants on days of blood acquisition. Baseline data will be collected by chart review. Cancer outcome data may be collected longitudinally by chart review only until the end of the study.

SUBJECT SELECTION & WITHDRAWAL

6- Inclusion and Exclusion Criteria

A. Inclusion Criteria

1. Advanced Metastatic NSCLC
2. Scheduled to initiate an immune checkpoint inhibitor
3. Age >= 18 years
4. Able to provide informed consent

B. Exclusion Criteria

1. Other, unrelated, concomitant active, invasive malignancy

7- Vulnerable Populations

Although vulnerable populations are not specifically excluded from this study, there will be no specific efforts to target these populations.

8- Subject Accrual

Eligible subjects will be identified by physicians, nurse practitioners, and physician assistants at the Abramson Cancer Center and PPMC. A total of 200 patients will be included in this study.

9- Subject Recruitment & Screening

All adult patients being evaluated by the Penn Thoracic Oncology group meeting inclusion/exclusion criteria will be screened for admission into the study. All prospective research subjects will meet with a study team member, the study will be explained, questions answered and the Informed Consent Process will be initiated

10- Data

This study will collect the following specific personal health identifiers (PHI) and other data elements for use at Penn. These data elements will be stored electronically in REDCAP, and will only be accessible to Penn study members. See **section 12** for specific data elements

INVESTIGATIONAL AGENTS

11. Drugs/devices (if applicable)

N/A

STUDY PROCEDURES

12. Procedures

1. Informed consent
2. Collection of baseline clinical information (chart review)
    a. Baseline demographic data (sex, race, ethnicity)
    b. Tobacco history
    c. Medical history
    d. Performance status
    e. Baseline tumor related variables, including histology, immunohistochemistry results, TNM stage, sites of tumor involvement
    f. Results of staging CT scans
    g. Mutation status as determined by clinical testing of tumor material
    h. Treatment regimens, including chemotherapy regimen, doses
3. Blood will be drawn once at baseline prior (up to 50 ml), and the frequency of research blood collection will not exceed the lesser of 50 mL or 3 mL/kg in an 8 week period and will not occur more frequently than 8 times per year. When possible, research blood samples will be drawn at the same time as the clinical sample to minimize discomfort and inconvenience to the patient. The listed time points below are approximations and will depend upon when the patient is having blood drawn for clinical purposes
    1. Baseline – up to 30 ml
    2. 2-4 weeks - up to 20 ml
    3. 8-12 weeks – up to 30 ml
    4. 14-16 weeks – up to 20 ml
    5. 18-24weeks – up to 30 ml
4. Subjects will be asked if they are willing to provide a single urine specimen in a sterile specimen cup at the time of enrollment
5. Although the vast majority of patients will have had tissue sequencing and immunohistochemical analysis to determine PD-L1 expression, if these analyses have not been performed, we may request that tumor tissue undergo this testing provided sufficient material is available. The result of this information may be disclosed to the patient and primary oncologist.
6. Longitudinal Data collection
    a. CT imaging to monitor treatment response
    b. Date of progression, and sites of progression.
    c. Date of death, if prior to end of study period
7. Analysis of blood and tissue samples. Samples may undergo the following analyses:
    a. Blood samples will undergo isolation of circulating free DNA and identification of specific mutations using either a PCR or sequencing approach
    b. Identification of specific proteins or cell surface markers using various protein measurement methods (e.g. mass spectroscopy, flow cytometry)

STATISTICAL PLAN

13. Sample Size Determination
This is a pilot analysis to determine the role of novel biomarkers for use as predictive markers in patients with lung cancer receiving immunotherapy. We are unable to conduct formal sample size analysis given the pilot nature of this study.

14. Statistical Methods
We will use descriptive statistics for variables collected. We will describe the demographic characteristics of subjects using frequencies and percentages for categorical variables and medians and ranges for quantitative variables. Data analysis will focus on the following analyses (based on our primary objective)

1. Prognostic markers: The analysis will determine the association with response rate, PFS, and OS.
2. When feasible, we will calculate the correlation between markers identified in the blood and in the primary tumor.

SAFETY & ADVERSE EVENTS

15. Potential Risks
The risk of study participation is minimal. The two main risks are loss of confidentiality and the risk of venipuncture. A number of mechanisms are in place to protect subject confidentiality (see above). The risk of venipuncture is minimal. There may be bruising or bleeding, and rarely infection. The overall amount of blood drawn for this study is not to exceed 50 ml not to exceed in an 8 week period and will not occur more frequently than 8 times per year and should not have any clinical implications for study participants.

16. Potential Benefits
Discovery of a minimally invasive biomarker to predict response to immunotherapy has the potential to significantly alter the current management of patients with lung cancer. This study may lead to the development of new methods for the management of patients with lung cancer receiving immunotherapy.

17. Confidentiality & Data Storage
Precautions will be taken to ensure that strict confidentiality is maintained. All source documents will be identified by a unique study ID number (unique for this project). No results will be reported in a personally identifiable manner. Study data will be maintained either on a study-dedicated computer whose files are password protected or in a locked filing cabinet to which only the investigators and their designated staff have access. Data that will be disclosed outside of Penn:

a. Baseline demographic data (sex, race, ethnicity)
b. Tobacco history
c. Performance status
d. Baseline tumor related variables, including histology, immunohistochemistry results, TNM stage, sites of tumor involvement
e. Results of staging CT scans
f. Mutation status as determined by clinical testing of tumor material
g. Treatment regimens, including chemotherapy regimen, doses

18. Data Safety & Monitoring

Safety data will be monitored by the principal investigator formally on a quarterly basis in the course of study conduct. We plan to have our data reviewed manually by the study coordinator who will bring any discrepancies to the attention of the study team for investigation and resolution.

19. Risk/Benefit Ratio
Although there is no immediate, direct benefit to the individual participants of this study, there is considerable potential benefit to future subjects, including participants in this study, and to the community as a whole. Physicians may be able to better predict the patients most likely to benefit from receiving immunotherapy for the treatment of lung cancer. In view of the minimal risks to study participants and considerable benefits of the study, the risk/benefit ratio is extremely favorable.

REGULATORY & ETHICAL CONSIDERATIONS

21. Informed Consent
Informed consent will be obtained during the patient's in-patient or out-patient visit at UPHS. Only competent patients will be approached to participate. This will be judged by an informal assessment by the investigator, but a mini-mental status exam will be performed if necessary. The goals of the study will be described by a study coordinator. The consent document will be reviewed and provided to prospective subjects. Comprehension of the consent document will be assessed by the study coordinator and investigators.

STUDY FINANCES

22. Funding Source
None

23. Compensation to Subjects for Participation
No compensation will be provided for the participants.

24. Conflict of Interest
All University of Pennsylvania investigators will follow the University conflict of interest policy. There is no conflict of interest for the principal investigator or any member of the study staff.

PUBLICATION PLAN

25. Publication Plan
Results of the proposed research may be presented at intramural conference sessions within the Abramson Cancer Center, and in various oral, print and electronic publications. The principal investigator holds primary responsibility for presentation of the results of the study.

References