Date: ____8-15-25_____
Sponsor: Kreftect, Inc._____

Protocol 001

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Title: Isotopic analysis to predict prostate biopsy outcome.

1. Abstract

The science of Earth history is driven by the method of isotopic fingerprinting, a highly sensitive methodology to track changes in the rate of chemical turnover even when it originates from a miniscule source in a much larger biological system. A tumor existing in the human body metabolizes elements at a different rate than non-cancerous tissue. The naturally occurring ratio of an elements heavy (more neutrons) and lighter isotopes (fewer neutrons) is altered as a result of transformations in tissue. These isotopes are utilized by metabolizing tissues at different rates. This altered metabolism than alters concentrations in serum of these elements as well as elements that serve as co-factors to key enzymes. These differences in serum element concentrations are then taken up by other tissues in the body that are proliferating / turning over. This includes hair, fingernails, and toenails. We have developed a method to detect the presence of a tumor in a person by doing an isotopic elemental analysis of hair. This method utilizes Inductivity Couple Plasma Mass Spectrometry (ICS-MS) of a hair sample with an AI algorithm to identify patients with cancer present by the presence of a unique set of elemental isotopes. Our preliminary results in mice demonstrate that hair strands robustly preserve isotopic signal that reflect prostate and breast cancer in mice. Our preliminary results demonstrate that hair strands robustly preserve isotopic signals that reflect the presence of prostate, kidney, and bladder cancers in patients.

Approximately one million prostate biopsies are performed per year in the United States alone, mainly driven by the finding of an abnormal PSA as part of prostate cancer screening. Approximately 250,000 men each year are diagnosed with prostate cancer as a result of these biopsies. In addition to this poor sensitivity and specificity, prostate biopsy is associated with significant infection and hospitalization risk (1-8). There is a clear unmet need to develop a test that would better predict the likelihood of prostate cancer on a biopsy.

Based on these results and the known power of the methodology, we here propose to demonstrate that isotopic analysis of hair can be utilized as a non-invasive test to detect cancer in men undergoing prostate biopsy.

2. Objectives (include all primary and secondary objectives)

The **primary** objective of this study is to develop a non-invasive test to detect prostate cancer, based on isotopic analysis of hair.

The **secondary** objective is to begin to determine if isotopic analysis can predict the grade of prostate cancer.

3. Background (briefly describe pre-clinical and clinical data, current experience with procedures, drug or device, and any other relevant information to justify the research)

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Short project description:

The need for better screening methodology to detect prostate cancer: The European Randomized study of Screening for Prostate Cancer (ERSPC), after a 16-year follow-up, showed that prostate-specific antigen (PSA) screening significantly reduces PCa mortality (1). Because PSA is elevated by many benign conditions such as prostatitis and benign prostatic hyperplasia, the sensitivity and specificity of PSA to detect cancer at biopsy is still far from optimal (2). The area under the ROC curve (AUC) for PSA to discriminate any prostate cancer vs no cancer is approximately 0.7 (2-5). Approximately one million prostate biopsies are performed per year in the United States alone, mainly driven by the finding of an abnormal PSA as part of prostate cancer screening (6). Approximately 250,000 men each year are diagnosed with prostate cancer as a result of these biopsies (7). In addition to this poor sensitivity and specificity, prostate biopsy is associated with significant infection and hospitalization risk (8). There is a clear unmet need to develop a test that would better predict the likelihood of prostate cancer on a biopsy.

Based on our preliminary data, we have developed a method to detect the presence of localized prostate cancer by performing an isotopic elemental analysis of hair. The purpose of this study is to determine if we can prospectively predict the presence of prostate cancer in men undergoing prostate biopsy because of an elevated PSA.

Introduction to isotopic fingerprinting: The science of Earth history is driven by the method of isotopic fingerprinting as a highly sensitive methodology to track changes in the rate of chemical turnover - even when it originates from a miniscule source in a much larger biological system. A tumor existing in the human body metabolizes elements at a different rate than non-cancerous tissue. This rate can be traced through changes in elemental abundances. Since isotopic alterations are reflected in the overall system of the human body, an elemental fingerprint can also be preserved in hair strands and nails – essentially acting as a "fossil record" of what is occurring in the entire body. Hair strands are known to preserve aspects of our lifestyles, such as diet or drug use, but are not yet used to reveal endogenous alterations that are associated with disease (9-13). Elements in the periodic table are isotopic if they contain varying numbers of neutrons in its core. Compared to few neutrons, a higher number of neutrons gives the isotope of that element a slightly higher weight. What makes isotopic elements useful for tracing chemical turnover are two things. First, isotopes of an element have slightly different chemical properties. Second, isotopes of an element occur at different abundances in nature, e.g., 32S (94.85%), 33S (0.76%), 34S (4.37%), and 36S (0.02%). The natural abundance of different isotopes can be altered as a result of tissue transformation where isotopes are utilized in a differential manner. When cells use isotopes of an element, their different weights associate with specific chemical bond strengths and therefore the energy needed to incorporate in the cellular process. Therefore, the use and re-use of light isotopes (few neutrons) will be in essence favored over the use of heavy isotopes (more neutrons). Altered metabolism within the body, as associated with tissue transformation, can alter the abundance of isotopic elements in both serum and key enzymes. These differences in abundances of different isotopic elements in serum are then taken up by other tissues in the body that are proliferating, including hair.

Preliminary data: Applying isotopic fingerprinting to the analysis of hair to detect cancer: We are developing a method to detect the presence of cancer and potentially other proliferative diseases in a person by doing an isotopic elemental analysis of hair. This has the potential to detect cancer in undiagnosed people (cancer detection screen) as well as monitor for the presence of cancer after therapy (cancer monitoring screen). This method combines Inductivity Couple Plasma Mass Spectrometry (ICP-MS) of a hair sample with an analysis of isotopic fractional abundances to identify patients with cancer present by the presence of a unique set of elemental isotopes. Our preliminary results demonstrate that hair strands robustly preserve isotopic signals that reflect the presence of cancer in mice bearing TRAMP prostate cancers or breast PyMT driven tumors as compared to control animals without cancer. Our further preliminary results demonstrate that hair strands preserve isotopic signals that reflect localized prostate, bladder, and kidney cancers as compared to controls. Fractional abundances of 72 isotopes and concentration values of 24 elements for 95 controls and 87 cancer patients (8 bladder, 8 kidney, 71 prostate) were utilized. Outlier values were detected using the interquartile range (IQR) rule and missing values were imputed using IterativeImputer (scikit-learn python implementation). Concentration data were normalized using row and column means. Data with near zero variance and highly correlated features were identified using DeepLINK (PMID: 34480002) and using SequentialFeatureSelector (scikit-learn python implementation). XGBoost analysis reached more than 80% accuracy with the test samples (14).

Based on these results and the known power of the methodology, we here propose to characterize how reflective isotopic fingerprints can serve as a diagnostic marker for prostate cancer. We will collect and analyze hair samples in men undergoing prostate biopsy as a result of elevated PSA. These samples will be collected prior to biopsy and run blinded to the biopsy result. Prediction by isotopic analysis will be compared to the biopsy pathology result to determine predictive power of isotopic analysis.

Expected results: We expect that, at a minimum, we will develop a non-invasive test to detect prostate cancer in men with an elevated PSA We will further define the suite of elements that are isotopically altered in the aspects of signal strength at different tumor burden, and which reflection signals that are robustly preserved, and can be analyzed, in hair.

Significance: The clinical significance of our vision is to create a diagnostic non-invasive tests for prostate cancer patients for early detection (The Isotect test). In the future we hope to create diagnostic non-invasive tests for all cancers

4. Study Procedures

a. Study design, including the sequence and timing of study procedures.

Study personnel will approach potential participants in cancer clinics who are scheduled to undergo prostate biopsy after obtaining permission from the physician. Potential participants will be asked to consider enrolling and providing a hair

specimen of ~5 grams (a bundle of ~50 hair strands; approximately a pencil width of hairs that are approximately 1/2 – 1 inch in length). Hair can be obtained from anywhere on the body and the root or follicle is not needed. It does not matter if the hair has been dyed or chemically treated. Participants will also be asked to provide a set of basic data: age, self-reported sex, self-identified race, state of residence and self-reported cancer history. Study personnel will receive permission to record medical record number and date of birth to track and record pathologic results of biopsies and subsequent surgeries if performed. Results will be anonymized prior to analysis.

b. Study duration and number of study visits required of research participants.

We expect that this study will involve collection of hair from participants over a 2 year period. We expect to collect at point of contact without any official study visits.

c. Blinding, including justification for blinding or not blinding the trial, if applicable. N/A

d. Justification of why participants will not receive routine care or will have current therapy stopped. N/A

e. Justification for inclusion of a placebo or non-treatment group. N/A

f. Definition of treatment failure or participant removal criteria. N/A

g. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely. N/A

h. If biological materials are involved, please describe all the experimental procedures and analyses in which they will be used. Analysis of isotopic compositions in hair are done with conventional mass spectrometry (IRMS).

5. Inclusion/Exclusion Criteria

Inclusion Criteria:

Patients who meet the following criteria are considered for inclusion in this study:

- Aged >40 years at time of consent.
- Intended to undergo prostate biopsy as a result of abnormal PSA
- Considered by a physician or healthcare provider as being at risk for PCa.
- Willing to consent to hair collection within 30 days prior to the date of the prostate biopsy
- Willing and able to participate in required study activities.

Exclusion Criteria:

- Current or past diagnosis of prostate cancer.