Title: Rapid methods (OncoSure ™) for isolation and profiling of Oncosomes for the early detection and monitoring the treatment of all human cancers from the peripheral blood.

Protocol Number:

Sponsor: Dr. Ramesh Babu

Contact info: email: rbabu@integenllc.com

Phone: 321-946-0403

Study background

Human cancers are of two forms - liquid and solid. There are only few screening tests for solid tumors such as PSA for prostate, mammograms for breast and colonoscopy for colorectal etc. The major drawbacks of the current systems are 1) longer TAT 2) higher cost 3) limited resolution for early detection of cancer 4) variability of sensitivity among different technologies to detect cancer 5) high infrastructure cost 6) Invasive procedures like tissue (for solid tumors) and bone marrow biopsy (for blood cancers). There is an urgent need for a noninvasive technique that has the following attributes: faster, more accurate, cost-effective and user friendly.

The proposed protocol solves the problem of the need to do 1) massive parallel sequencing of the genome to detect cancer specific abnormalities 2) to apply ultracentrifugation technologies to obtain extracellular vesicles produced by cancer cells 3) sophisticated and expensive imaging technologies such as CT, MRI and PET scans to identify cancer nodules

This protocol (invention) is an improvement on what currently exists. The invention is different from the existing sequence-based methods because it employs rapid in situ hybridization platform and DNA fluorescent probes for ploidy detection. The invention is different from the existing gold standard of ultracentrifugation for the separation of extra cellular vesicles produced by the cancer cells, by employing an inexpensive density gradient and sequential low speed centrifugations.

The proposed method is better than the existing methods because of 1) fast TAT 2) lower cost 3) ease of use in almost all laboratory setups without significant infrastructure expenditure 4) comprehensive screening for aneuploidy of all chromosomes and 5) detection of all human cancers at an early stage.

Objectives

1) to conduct a proof-of-concept study that has the components of demonstrating a) a rapid non-invasive method of isolating the Oncosomes, the extra cellular vesicles produced by the cancer cells, from human plasma, b) an ultra-fast in situ hybridization protocol with specialized buffers and multiplex-labelled DNA fluorescent probes c) correlation of presence/absence and quantitation of Oncosomes with the presence/absence of cancer

2) to conduct a triple-blind clinical study to validate that it is possible to detect the presence of any human cancer from a liquid biopsy, using aneuploidy the hallmark of the human cancer by combining the efficient isolation of the Oncosomes from the plasma, with rapid hybridization of the fluorescent DNA probes with multiplex labeling scheme.

Scientific background

There are many ways to obtain plasma from a suitably collected blood sample. The simple and easy way is simply let the blood sample stand still upright for few hours and the top layer will be the plasma. However, for a rapid turnaround addition of a density gradient followed by centrifugation would accelerate the process of obtaining plasma. There are many density gradients commercially available for separation of blood components. For this protocol, LymphoPrep was chosen to efficiently isolate the plasma.

The field of Exosomes is a decade old and numerous technologies have been developed during this period to isolate exosomes, which are the smallest of the extracellular vesicles produced by various cells in the body. A partial list of these technologies includes Ultracentrifugation, Size Exclusion Chromatography (SEC), Ultra filtration, Various precipitation methods, immuno magnetic separation etc.

The cargo of exosomes includes various proteins, mRNA, miRNA and few other macro molecules. Numerous researchers have used different components of this exosome cargo in their efforts to develop assays for detecting solid tumors from liquid biopsy. Very little attention has been focused on the largest of the exosomes, namely Oncosomes which are ONLY produced by the cancer cells and contain whole genomic DNA, whereas exosomes contain very little if any of genomic DNA. Since this protocol relies on the detection of ploidy, which is based on the whole genomic DNA, Oncosomes are the only suitable extracellular vesicles to determine the aneuploid status which is the hallmark of all human solid tumors. Therefore, if one can successfully isolate and characterize the Oncosomes, one can detect the cancer from liquid biopsy. The simple sequential low to medium speed centrifugation steps utilized in this protocol guarantee the maximum recovery of Oncosomes.

The common practice in the diagnostic workup of in situ hybridization is to use one or just a few, generally less than four, fluorescent DNA probes to investigate specific targets in the genome. This approach falls way short of obtaining the entire ploidy information in a single assay. The multiplex approach utilized in this invention guarantees that each Oncosome can be queried for its ploidy status in a single assay with 12 chromosomes assayed in each chamber on the slide and by combining the results of the two chambers, one can get the ploidy status of the whole sample in a single assay without the need for any special equipment and software than a simple fluorescent microscope equipped with few filter cubes to detect all colors emitted by the multiplex DNA probes.

Turnaround time (TAT) plays a critical role in any diagnostic workup. Historically in situ hybridization protocols utilized an overnight time period to get the results. In the current invention a rapid hybridization protocol is utilized with results in as little as 15 min to less than an hour. Combining the efficient separation of plasma, maximum recovery of Oncosomes, multiplex DNA probe labelling and rapid in situ hybridization technology one can successfully determine the presence or absence of a cancer from a noninvasive sample collection such as a peripheral blood in less than three hours.

The protocol utilizes DNA fluorescent probes for landmarks on all 24 human chromosomes and rapid in situ hybridization[1,2] to determine the ploidy of the Oncosomes. Since Oncosomes are ONLY produced by cancer cells[3] and they mimic parental genome[4], the invention utilizes the “hallmark” of all cancer cells i.e., aneuploidy[5] in detecting the presence of all human cancer(s) via the peripheral blood, the so-called liquid biopsy. The protocol is different from the gold standard of ultracentrifugation to isolate the extra cellular vesicles produced by the cancer cells, by employing an inexpensive density gradient[6] and sequential low speed centrifugations. The existing methods depend on genome sequencing, which has method failure rates ranging from 5-10%. Ultracentrifuges are very expensive instruments and only few

research and commercial laboratories have the necessary infrastructure. Additionally, all current assays concentrate on the smallest exosomes which have only trace amounts of genomic DNA[7] and therefore are unsuitable for profiling the hallmark trait i.e., the aneuploidy, of the cancer cells.

References

1 Babu R, Van Dyke DL, Papa S, Fuentes E, Fuentes S, Kopuri S, Williamson C, Liu M, Dev VG, Tepperberg J, Schwartz S, Papenhausen P and Koduru P. Development and Validation of 15-minute FISH hybridization technology for interphase and metaphase cytogenetic samples. Platform presentation at American Cytogenetics Conference, June 2018

2. Ramesh Babu 15-minute InstaFISH. Workshop and Live Demonstration. Cancer Genetics Consortium Annual Meeting, August 2018

3. D. Di Vizio, et al., Large oncosomes in human prostate cancer tissues and in the circulation of mice with metastatic disease, Am. J. Pathol. 181, 1573–1584, 2012

4 Nobuyoshi Kosaka, Akiko Kogure, Tomofumi Yamamoto, Fumihiko Urabe, Wataru Usuba, Marta Prieto-Vila, and Takahiro Ochiya . Exploiting the message from cancer: the diagnostic value of extracellular vesicles for clinical applications. Experimental & Molecular Medicine 51:31, 2019

5 Kristin A. Knouse, Teresa Davoli,Stephen J. Elledge,and Angelika Amon. Aneuploidy in Cancer: Seq-ing Answers to Old Questions Annu. Rev. Cancer Biol.1:335–54, 2017

6 Lymphoprep™ Density gradient medium for the isolation of mononuclear cells. STEMCELL Technologies Inc.

7 Tatyana Vagnera, Cristiana Spinelli, Valentina R. Minciacchi, Leonora Balajf , Mandana Zandiana, Andrew Conleyb , Andries Zijlstrag , Michael R. Freeman, Francesca Demichelisi , Subhajyoti Dej , Edwin M. Posadask , Hisashi Tanakaa,and Dolores Di Vizio .Large extracellular vesicles carry most of the tumour DNA circulating in prostate cancer patient plasma J Extracell Vesicles.7(1): 1505403, 2018

The research hypothesis

By combining the efficient isolation of the Oncosomes from the plasma, with rapid hybridization of the fluorescent DNA probes with multiplex labeling scheme, it is possible to detect the presence of any human cancer from a liquid biopsy, using aneuploidy the hallmark of the human cancer.

Design and methodology

Product Overview/Test Principle – various components

1. Obtain peripheral blood and separate plasma using the density gradient LymphoPrep, if needed use stabilizers for transport

2. Separate cell debris and apoptotic bodies by low-speed centrifugation of plasma at 2800 g, Save supernatant.

3. Isolate Oncosomes by pelleting by centrifugation at 9000 g. Discard supernatant.

4. Suspend the Oncosome pellet in PBS and harvest the cell button by hypotonic (KCl) and fixative treatments freshly prepared Methanol: Acetic acid 3:1 ratio

5. Drop the resuspended pellet solution onto two chambers on a glass slide
6. Prepare or obtain from InteGen LLC, Fluorescent labelled DNA probes for specific genomic targets on individual chromosomes such as telomeres, centromeres, and specific loci
7. Perform rapid in situ hybridization using the fluorescent DNA probe mixes on each chamber respectively, for ploidy detection
8. Enumerate the number of Oncosomes in each chamber
9. Determine the ploidy status of the Oncosomes in each chamber
10. Complete the analysis a) Presence or absence of Oncosomes b) Enumeration (quantitation) of Oncosomes c) Ploidy status Normal or abnormal, of Oncosomes

Relationship Between the Components:

Step 1 is a pre-requisite for step 2. In the first step, the plasma is separated from the whole blood and in the next step, the cell debris is removed. Oncosomes are isolated in step 3. Step 4 is an integral part of step three, where the Oncosomes are prepared for further characterization. Step 4 is a pre-requisite for step 5 where the cell suspension is deposited onto the glass slide. In Step 6, Fluorescent labelled DNA probes are made or obtained from InteGen LLC for specific genomic targets on individual chromosomes such as telomeres, centromeres, and specific loci. This is a pre-requisite for rapid FISH hybridization which is carried out in the next step 7. Enumeration of Oncosomes is carried out in step 8 for each chamber on the glass slide. In step 9, the ploidy status is determined from the DNA probe hybridization results. In the first chamber the ploidy status for chromosomes 5, 7, 8, 13, 15, 16, 17, 18, 21, 22, and sex chromosomes (X and Y) is determined and in the second chamber the ploidy status for chromosomes 1, 2, 3, 4, 6, 9, 10, 11, 12, 14, 19 and 20 is established. Finally in the last step (step 10), the analysis is completed by documenting the enumeration status as well as the ploidy status of the Oncosomes. Based on the results of this analysis, a determination of whether a tumor exists in the body is made.

Data analysis

INTERPRETATION OF RESULTS

1. Clear separation of plasma from blood after the use of LymphoPrep assures that the extra cellular vesicles are separated from regular nucleated cells such as white blood cells
2. Sequential differential centrifugation ensures that the largest exosomes i.e., Oncosomes are separated from contaminating cellular debris and smaller exosomes, into the correct cellular fraction
3. Hybridization with DNA probes which results in emitting fluorescent signals signifies the presence of Oncosomes
4. Absence of any fluorescent signals indicates no Oncosomes
5. Enumeration/quantification of the Oncosomes i.e., 7 or less Oncosomes is interpreted as normal range and absence of cancer or genetic remission in a patient with history of cancer and on treatment
6. Eight or more Oncosomes is considered a POSITIVE result for the presence of a malignancy, either a solid cancer or hematologic malignancy

Study procedures

Description of Steps and Sequences Used in the OncoSure™ Test:

There are basically four steps critical for this protocol 1) Efficient separation of plasma from the suitably collected blood sample 2) Better isolation and enrichment of Oncosomes from the plasma 3) Multiplex fluorescent labelling of DNA probes and 4) rapid in situ hybridization.

Several vacutainer tubes are commercially available for drawing the peripheral blood. For the purpose of this protocol, only tubes with a proper anti-coagulant so that plasma can be separated will be used. There are many suitable blood collection tubes for this purpose such as EDTA purple or lavender tubes. Tubes designed for serum collection should be avoided. These generally have red or black tops. There are many density gradient solutions available. But the Lymphoprep with a density of 1.077 g/ml is best suited for efficiently separating the plasma as well as buffy coat which retains all mononuclear cells including the tumor cells, at a centrifugation of 1200 g for 10 minutes at room temperature. Since the Oncosomes are smaller than any of the mono nuclear cells including the tumor cells, they float to the top layer (plasma) after the density gradient centrifugation. In order to get rid of cell debris an initial centrifugation at 2800 g for 10 min, followed by a slightly higher centrifugation of the supernatant at 9000 g for 15 min will pellet the Oncosomes leaving all other exosomes in the supernatant. One can get the Oncosomes by resuspending the pellet in PBS. To process them further for characterization, the traditional cytogenetic method of fixation using freshly prepared methanol: acetic acid with a 3:1 ratio is employed. Once the Oncosomes are fixed, the suspension is dropped onto a glass slide in two separate areas/chambers for in situ hybridization. There are many commercial sources for obtaining BAC probes and for this invention BACPAC library resource[8] is utilized to get the needed BACs. To generate the BAC DNA, several commercial BAC extraction protocols are available including propagation of BAC cultures. Labeling of BAC probes can be done by many protocols available, but for this protocol Nick translation will be utilized with various fluorescent dNTPs.

In order to study the genome ploidy of each Oncosome present in each of the chambers on the glass slide, a multiplex hybridization protocol will be utilized as detailed In Table 1. There are many in situ hybridization protocols in the public domain and most employ an overnight hybridization time. Some vendors claim rapid hybridization from one to several hours. The procedures employed in this protocol with specialized hybridization buffers allow a very rapid hybridization with results in as little as 15 minutes to less than an hour[1,2].

Table 1

	PL1	PL2
Green	13q11, 16q11	3p11.1, 19q11
Green Hybrid	Yq11.1	6q11.1
Red	21q22.13	9q34
Red Hybrid	Xp11.1	11q11
Red/Green Hybrid	18q11.1	10q11
Gold	22q11, 15q11	1q12, 20q11
Gold Hybrid	5q11	4q11
Aqua	7q11	2q11.1
Aqua Hybrid	8q11	12p11.1
Gold/Aqua Hybrid	17q11	14q11

Reference

8. BACPAC library. https://bacpacresources.org/

Study Design: Proof-of-Concept (POC) Study

Approximately 100 subjects, about half healthy controls without any history of cancer and 50 subjects with either an active cancer, or history of cancer or on a treatment post diagnosis of a cancer will be recruited. The participating investigators are physicians from various disciples such as family practice, oncology etc. The geographic locations can be any place within the United States. A small (2-3 cc) peripheral blood sample will be sent to the testing facility in Orlando, the location of the Sponsor (Dr. Babu). An informed consent, either verbal or a simple one-page form will be used from each subject. Investigators will be given a summary of the study and blood collection procedure. This POC study will be conducted in a "semi-blind" fashion with the objectives of making sure several forms of cancer patients are included and the test is capable of disease detection and monitoring, by establishing the normal ranges. All patient data will be kept in the Sponsors office and the results discussed in a deidentified manner such that the patient identify is kept confidential.

Clinical Validation triple blind study

Approximately 400 study subjects, about half cancer patients and half controls will be enrolled. Participating physician investigators can be associated with individual practices, cancer treatment facilities or hospitals etc. The geographic locations can be anywhere in the United States.

Study protocol:

1. An IRB approved informed consent will be used to recruit the subjects into the study
2. An alphanumeric string supplied by the sponsors will be placed on the blood sample tube with no other information, from the participating physician practices
3. The sample will be sent to InteGen LLC, the Sponsors location in Orlando

4. The alphanumeric string with patient demographics and clinical history will be sent by the investigators to an independent third-party study coordinator who has agreed to strict confidentiality and nondisclosure of results to any party until the entire study is complete
5. The testing facility (Sponsor) will send results with attached alphanumeric string to the study coordinator
6. Once the study coordinator has been informed that the study is complete, the coordinator will match the clinical data and the laboratory testing data with the alphanumeric string and generate a master study results EXCEL spread sheet
7. The master EXCEL spread sheet will then be shared simultaneously to the Sponsor and to the participating investigators.
8. Neither the sponsor nor the participating investigators are allowed to alter the master results database
9. Any perceived discrepancies will be clarified in coordination with the original database kept by the study coordinator.
10. The results will be decoded and analyzed for specificity, sensitivity, and overall accuracy of the test OncoSure™ in detecting and monitoring human malignancies noninvasively from the peripheral blood.

Study Recruitment

Participating physician investigators will explain the rationale of the study to their patients and non-cancer healthy adults to recruit the subjects into the study. Willing participants will sign the IRB informed consent form.

Details about the subject population

The POC study predominantly consists of adult subjects. Physician investigators will select subjects comprising various forms of cancers, solid as well as hematologic and at various stages in their treatment plan. Generally, subjects reside in the same area as the clinics but could come from a different geographic location than the participating clinic. For some cancer types, there may be predominance of one sex or other, but the investigators will attempt to recruit both sexes consistent with the prevalence of that cancer. The POC study may last for 2-4 weeks.

For clinical validation blind study both pediatric and adult cancer patients and healthy controls will be recruited. Same characteristics discussed for POC study also apply here. The differences are 1) use of IRB approved consent form 2) coordination with the independent study coordinator. The blind study may last for 4-8 weeks.

Subject Inclusion Criteria

An individual must meet the criteria below to be eligible.

- Individual is a patient or affiliate of the participating physician practice
- Any patient diagnosed with any cancer prior to start of therapy

- Any cancer patient at any stage of treatment
- Any patient suspected of a cancer or a history of cancer
- Individual is 2 years or older
- Individual is willing to participate in study procedures and able to provide written or verbal informed consent for the POC study and the IRB approved informed consent for the clinical validation blind study in the English language.

Subject Exclusion Criteria

An individual cannot meet the below criteria.

- Individual is unable to provide written informed consent in the English language.

Subject Withdrawal/Discontinuation Criteria

Subject may withdraw consent for any reason at any time. Depending on the timing of withdrawal of consent, specimens/data might never be collected from the subject. If specimens were collected and tested or if data were collected prior to withdrawal the specimens and data may have been used. Data and leftover/remnant samples from withdrawn individuals will not be used in future research.

In addition, the investigator may discontinue a subject's participation. Reasons for withdrawal may include:

- Subject was erroneously enrolled and did not meet eligibility criteria.
- Subject did not provide any specimen(s).
- Subject failed to comply with study procedures.

A subject may be withdrawn for reasons other than those stated above/ If so, then the specific reason will be recorded.

Individuals who withdraw will have the following data recorded/

1. Date of withdrawal.
2. Reason for withdrawal.

Collection Site Procedures

- The subject will document informed consent and will be enrolled.
- The collection site will use the alphanumeric string (ID) provided to them by the sponsor for the clinical validation study
- At specimen collection, the collection site will verify informed consent is documented and obtain a 2-3 cc blood sample and send it to the Sponsor's testing lab
- For clinical validation study, clinical data on each participant will be sent to the study coordinator with corresponding alphanumeric string

- For POC study clinical data may be provided along with specimen to the testing lab

Informed Consent

Interested individuals will be provided the ICF. The ICF will be written in English using nontechnical language whenever possible. Additional documentation may be provided if required by the IRB or applicable regulation. Collection site study staff will review the ICF, allowing sufficient time for the individual to ask questions. Consent will be documented when the individual agrees to participate and personally signs and dates the ICF.

The ICF will include the required elements in accordance with GCP and applicable regulations. The sponsor will develop the ICF.

The collection sites will obtain and document informed consent in accordance with 21 CFR part 50, any other applicable regulatory requirements, and GCP. The collection sites will provide the IRB-approved ICF to potential study subjects who will be given sufficient opportunity to read, understand, ask questions, and consider participation. All subjects participating in the study must give be given sufficient opportunity to read, understand, ask questions, and consider participation. Informed consent will be documented by the subject's signature and date in accordance with 21 CFR part 50. The subject will be provided with a copy. The signed original ICF will be kept by the collection sites in a secure area with access limited to authorized study team members.

Subject Identification

After the subject documents consent, the subject is enrolled and assigned a subject ID. Unique specimen IDs will be assigned for each specimen collected. Specimen IDs will be linked to the appropriate subject ID. All IDs for the study will not contain any personal identifying information.

Specimen Collection

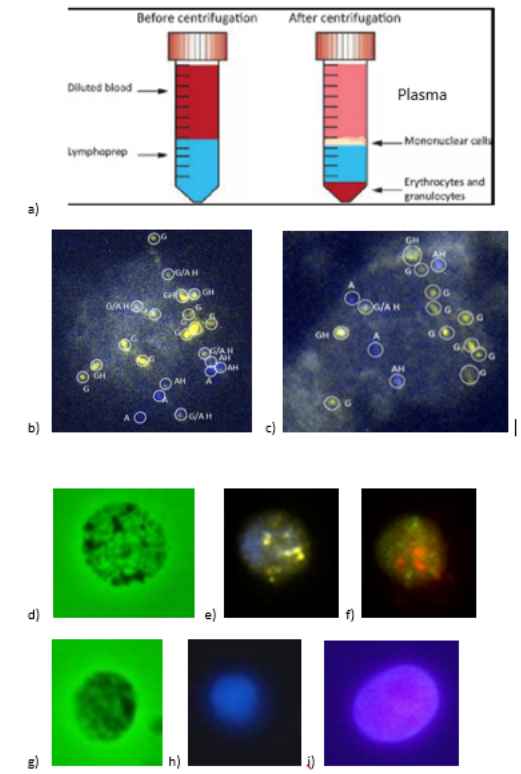
2-3 cc of peripheral blood will be collected.

INTERPRETATION OF RESULTS

1. Clear separation of plasma from blood after the use of LymphoPrep assures that the extra cellular vesicles are separated from regular nucleated cells such as white blood cells
2. Sequential differential centrifugation ensures that the largest exosomes i.e., Oncosomes are separated from contaminating cellular debris and smaller exosomes, into the correct cellular fraction
3. Hybridization with DNA probes which results in emitting fluorescent signals signifies the presence of Oncosomes (See Figure 1)
4. Absence of any fluorescent signals indicates no Oncosomes
5. Enumeration/quantification of the Oncosomes i.e., 7 or less Oncosomes is interpreted as normal range and absence of cancer or genetic remission in a patient with history of cancer and on treatment

6. Eight or more Oncosomes is considered a **POSITIVE** result for the presence of a malignancy, either a solid cancer or hematologic malignancy

Figure 1



Legend: a) Cartoon showing the blood components before and after centrifugation with Lymphoprep. Oncosomes will float into the plasma

b) tumor cell and c) Oncosome from the tumor cell, both showing similar genome profile after in situ hybridization with DNA probes

d) A tumor cell, e) and f) Oncosomes produced from the tumor cell from K562 cell line, all at 60X magnification. Notice the size difference between parental tumor cell and the Oncosomes

g) A tumor cell from a solid cancer patient and h) Oncosome from the same patient both at 20X magnification. Notice the size difference between the tumor cell and the Oncosome

I) Tumor cell from SKBR3 a breast cancer cell line, at 20X magnification shown as a reference

Sample Size Justification

One of the objectives of the study is to achieve a specificity of 95% and a sensitivity of 95%. This means that a 5% false positive and negative, respectively, will be acceptable to FDA. With approximately 250 cancer patients and 250 non-cancer patients, it would take 13 false negative and 13 false positive cases to make the proposed test unreliable as a screening test. Based on the preliminary results, it seems highly unlikely that there would be that many false positives and negatives.

Therefore, a sample size of 500 is well justified.

Ethical Conduct of the Study

All parties involved will be responsible for ethical conduct of this study and will comply with recognized ethical principles for medical research involving humans and the principles of GCP and regulatory requirements that are applicable to in vitro diagnostic device clinical trials.

Institutional Review Board Review

Before study activities are initiated, the study sponsor will obtain written and dated approval from the central IRB for the protocol and any other relevant documents (e.g., ICF) in accordance with the requirements in 21 CFR part 56.

During the study, the sponsor will obtain central IRB approval of any amendments to an IRB-approved document (e.g., protocol, ICF). The sponsor will ensure reports are submitted (e.g., protocol deviations) to the central IRB, per IRB requirements. The sponsor will ensure continuing review of the study by the central IRB, if applicable.

After the study is completed, the sponsor will provide the central IRB with any required documentation such as a final report per the IRB's requirements.

If the collection site requires review and approval from their local IRB, the investigator will be responsible for ensuring IRB review and approval for applicable documents.

Expected Benefits and Risks

Cancer is a major health concern around the world. Most cancers are detected when they are at a much-advanced stage. Early detection provides the opportunity to treat the cancer resulting in better survival rates. Unfortunately, there are screening tests for only few cancers and even these tests are associated with high false positive and negative rates.

The major drawbacks of the current testing methods are 1) longer TAT 2) higher cost 3) limited resolution for early detection of cancer 4) variability of sensitivity among different technologies to detect cancer 5) high infrastructure cost 6) Invasive procedures like tissue and bone marrow biopsy

The proposed study with the aim of developing a screening test, OncoSure ™ solves the problem of the need to do 1) massive parallel sequencing of the genome to detect cancer specific abnormalities 2) to apply ultracentrifugation technologies to obtain extracellular vesicles produced by cancer cells 3) sophisticated and expensive imaging technologies such as CT, MRI and PET scans to identify cancer nodules.

OncoSure ™ is an improvement on what currently exists. The test is different from the existing sequence-based methods because it employs rapid in situ hybridization platform and DNA fluorescent probes for ploidy detection. The test is different from the existing gold standard of ultracentrifugation for the separation of extra cellular vesicles produced by the cancer cells, by employing an inexpensive density gradient and sequential low speed centrifugations. It is better than the existing methods because of 1) fast TAT 2) lower cost 3) ease of use in almost all laboratory setups without significant infrastructure expenditure 4) comprehensive screening for aneuploidy of all chromosomes and 5) detection of all human cancers at an early stage.

Study participation has minimal risk. Collection of a small amount of blood (2-3 cc) is generally simple and has very minimal discomfort. Complications are uncommon and would be minimal, such as redness at the site of needle insertion.

Results from the OncoSure ™ test will be provided to subjects. Subject communications will note that the test is not authorized or approved for screening and that further evaluation is necessary to determine cancer status or efficacy of the cancer treatment.

False positive and negative results may occur. No medical treatment or guidance on treatment decisions will be provided. Thus, there are no anticipated health benefits to the subject for participating in this study. Information gained from evaluation of the study specimens may support the development of a new product, in this case a screening and a monitoring test which could potentially affect care given to cancer patients in the future.

Confidentiality and Anonymity

The investigators, site study staff, study coordinator, and sponsor will prevent disclosure of the subject's identity to unauthorized parties/individuals.

Signed consent forms will be stored in a secure location. These files will be made available to authorized sponsor, IRB, or regulatory authority personnel for monitoring and/or auditing purposes.

De-identified subject IDs will be linked to subjects; this key will be held by the study coordinator. The de-identified subject IDs will be assigned to the subject upon enrollment. All specimens and study data will be identified with study subject or specimen IDs, as appropriate.

Any other study records provided to the sponsor will be stored in a secure location with access limited to the PI. Study files will be made available to the IRB, regulatory authorities, and/or sponsor personnel should they request access for auditing purposes.

Only the PI will have access to the complete information by a password to the database and strict confidentiality will be maintained by the PI and no identifiable data will be released by PI to anybody including FDA.

Unanticipated Adverse Device Effects

An unanticipated adverse device effect (UADE) is any serious adverse effect on health or safety, or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

The testing site will report all UADEs to the sponsor. The sponsor will evaluate all reported UADEs in accordance with PI-provided work instructions and terminate the study, or portions of it, as soon as possible if that effect presents an unreasonable risk to users. UADEs will be reported to the FDA, IRBs, and investigational test sites in accordance with applicable guidance and regulations.

Compensating Research Subjects

All study subjects will participate in the study voluntarily. A token compensation of $50 will be provided to cover their transportation costs.

Selection of Investigators and Sites

Investigators will be responsible for fulfilling the clinical study requirements as specified in this clinical protocol and per applicable GCP requirements and country specific regulations. The study site must have the necessary resources to comply with these requirements. Sites will be selected using criteria outlined in the sponsor's WI, Site Qualification Visit and Selection.

Training

Prior to starting study activities, collection and testing site study staff will be trained on the protocol and other study instructions, and the investigational device, as appropriate to the role in the study. Training will be documented on training logs.

OncoSure™ test will be done as per the established procedures by qualified research staff at Sponsors testing facility.

Note: If new site study staff are assigned to the study after the initial training, the site must contact the clinical study monitor to coordinate training.

Protocol Deviations

Every effort must be made to ensure that study conduct is performed in accordance with the study protocol, other study instructions, and training provided. Deviation reporting will follow guidelines from the IRB/IEC. Under no circumstances should sampling handling methods or techniques deviate from those specified in this study protocol or from established procedures defined by the sponsor. Deviations would compromise the integrity of the data. Should a deviation occur, it must be documented and explained on the protocol deviation reporting form and reported to the clinical study monitor.

Protocol Amendments

Amendments to this study or protocol may be made during the study and must be approved by the sponsor and the IRB. The site study staff must be trained to the protocol amendment before the amended protocol is implemented.

Source Data/Documents

The investigator will maintain adequate and accurate source documents and study records that will be retained at the site. Source documents may include laboratory notes, memoranda, recorded data from instruments, film, or other digital images that have been verified as being accurate copies.

Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Any changes to source data will be made in accordance with good documentation practice and will be traceable, will not obscure the original entry, and will be explained if necessary (e.g., via an audit trail).

Study Monitoring and Audits

The sponsor will monitor the study to ensure the study is conducted in accordance with the protocol, GCP, and applicable regulations. Monitoring may include onsite and remote data review. The sponsor will monitor completed ICFs to ensure appropriate documentation of informed consent and perform source data verification.

During and after the study, sites must accommodate study-related monitoring, audits, and inspections by the sponsor, IRBs, and appropriate regulatory authorities, including providing direct access to source

documents or data. The investigator must notify the sponsor immediately upon becoming aware of an audit or inspection performed by regulatory authorities.

Financial Disclosure

The investigator(s) participating in this study are required to provide sufficient and accurate financial information to the sponsor pertaining to:

1. Any arrangements between the sponsor and the investigator whereby the value of compensation to the investigator for conducting the study could be influenced by the outcome of the study;

2. Any significant payments or other sorts of compensation from the sponsor, such as grants to fund ongoing research, compensation in the form of equipment, retainers for ongoing consultation or honoraria;

3. Any proprietary interest in the product to be developed and held by the investigator involved in the study; and

4. Any significant equity interest in the sponsor held by the investigator involved in any study.

The investigator shall promptly update this information if any relevant changes occur during the investigation and for up to 1 year following completion of the study.

Termination of the Study

The sponsor and the investigator, institution, or regulatory agency have the right to terminate the study at their discretion with written notice to the other parties. This action should be taken only after appropriate consultation between the sponsor and the investigator or institution.

Investigator Responsibilities

The investigator shall be responsible for the day-to-day conduct of the study. The investigator will ensure that the study is conducted in compliance with GCP, the study protocol, and applicable regulatory requirements.

Sponsor Responsibilities

The sponsor has the overall responsibility for the conduct of the study, including assurance that the study meets the applicable regulatory requirements.