

Study Protocol

Study Name: Genomic and Imaging Study for Patients Undergoing Surgery for Liver Cancer

Principal Investigator: Sandi Alexander Kwee, MD

Primary study site: The Queen's Medical Center, Honolulu, Hawaii

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Detailed Study Protocol

I.1. Study Protocol Schedule

Enrollment:

- Signed consent form and completed eligibility checklist (Oncology Research Department (ORD) Nurses to complete Patient Selection Criteria and Enrollment).
- Relevant clinical documentation is reviewed by the PI (Dr. Kwee) prior to authorization to schedule PET/CT appointment.
- Serum pregnancy test is performed (if appropriate).
- PET/CT appointment is scheduled.

Imaging Appointment (1.5h):

- PET/CT using Liver F-choline Scan Protocol within 14 days of surgery.

Day of Surgery:

- UHCC Pathology Shared Resource personnel are notified and available for specimen procurement.
- Specimen obtained under UHCC Specimen Procurement Standard Operating Procedure (see Appendix following this Protocol).

Follow-up

- Blood tests which include a comprehensive metabolic panel (includes liver function and chemistry panel), protime and complete blood count will be drawn at the subject's 1-4 week follow-up visit with their surgeon. These tests will be performed by a certified diagnostic laboratory such as DLS. If subject agrees, an optional research blood sample will also be collected and sent to UHCC for analysis.
- Subjects are also enrolled into the Hawaii Tumor Registry. Therefore, the ORD staff will not be involved in extended follow-up data collection, given that this falls within the scope the State-mandated tumor registry activities.

I.2. Criteria for Patient Selection

Inclusion Criteria: **Inclusion Criteria:** 1) > 18 years of age. No upper limit of age. 2) liver tumor diagnosed histologically as HCC or suspected of being HCC in association with serum alpha-fetoprotein level > 200 or tumor mass with characteristics of malignancy on diagnostic imaging, 3) under the care of a surgical attending, 4) deemed a surgical candidate and has agreed to surgery to remove a portion of the liver containing tumor, and 5) Child-Pugh A/B.

Exclusion Criteria: 1) Weight > 350 lbs (PET/CT limit); 2) pregnant or lactating female, a serum pregnancy test will be performed within 2 weeks or less before the date of the FCH PET/CT scan in all women capable of becoming pregnant; 3) serious underlying medical condition that would impair patient's ability to tolerate the imaging procedure; 4) concurrent treatment with chemotherapy, molecule-selective, biological, or radiotherapeutic agent.

I.4. Method of Enrollment and Follow-up

Written informed consent will be obtained by the PI or other physician listed on FDA Form 1572. The Research Nurse (Karen Ng or other ORD nurse) or Associate (Miles Sato or other ORD Associate) will assist the PI with recruitment, enrollment, scheduling, and follow-up.

If the subject experiences a serious adverse event, follow-up of the adverse event to its conclusion will ultimately be the responsibility of the PI and the IND sponsor-investigator (who serves as co-investigator on this study). The patient's treating physician will be allowed to assist with clinical follow-up.

I.4.1. Authenticating Disease Progression

Clinical information (test results, etc.) will be reviewed for the purpose of confirming inclusion/exclusion criteria.

Evidence of disease progression will be collected and made available for descriptive purposes and retrospective analyses. Data collection to support authentication of disease progression will include documentation of patient demographics, age, performance status, clinical stage, details and dates of the primary therapy, and post-treatment clinical status. The presence of potential disease sites will be documented based on reports of any available imaging studies including but not limited to CT, MRI, ultrasound, and bone scan. This data will be assembled on study specific forms by the personnel for this study. The PET image data and genomics data are high-dimensional datasets. This type of data will not be available in study forms but will remain in their source format. The survival data will also not be collected by study personnel or entered into the study specific forms, since they will be obtained directly from the Hawaii Tumor Registry through query reports.

I.5. Method for Determining Doses, Maximum dosage, and Duration of Patient Drug Exposure

Patient dose has been determined by the least radiation dose from the radiopharmaceutical that will provide enough counts in the tomograph detectors to obtain quantifiable images with adequate counting statistics. The exposure of the patient to the radiopharmaceutical will only be until the radioactive label decays. The half life of fluorine-18 is 109.8 minutes. Maximum duration of radiation exposure is therefore limited by this half-life. The number of doses administered is determined by the number of PET/CT scans required by the study protocol. The total effective dose will not exceed reasonably safe limits set by the FDA as described in the investigator's brochure for 18F-choline. Each dose of 18F-choline is administered intravenously as a single unit dose. A radioactivity dose of 0.09 millicuries per kilogram of body weight will be prescribed and obtained from the cyclotron laboratory. A dose calibrator in the PET imaging suite will be used to confirm the actual dose to be administered. Only a dose falling within a 10% range of the calculated prescribed dose will be administered.

I.6. Observations and Measurements to Fulfill the Objectives of the Study

Source documents (clinical, radiographic, and pathology reports), obtained from the medical record (office or hospital) following subject written informed consent, will be abstracted to generate a list of known sites of active disease. A table of these sites for each subject will be entered into a password-protected database.

I.7 Investigational Observations and Measurements to Fulfill the Objectives of the Study

The 18F- Choline injection is infused into the subject intravenously over 1 minute. The PET scanning is started approximately 10 minutes after the start of infusion. PET imaging data, from the neck to the proximal femur is then collected. PET data is analyzed by: a) visual assessment, and b) semi-quantitative region of interest (ROI) analysis. Standardized uptake values (SUV) will be recorded from each ROI. Implicit in the definition of SUV is the requirement that the tomograph be properly calibrated so that activity in tissue can be determined in uCi/ml. Structural imaging (CT, MRI) when available will be used for anatomical correlation. If a PET/CT scan is performed, the CT performed for attenuation correction will provide some anatomical correlation data. The PET/CT imaging protocol is described in section I.10.

Patients will routinely schedule follow-up visits with their surgeons at 1-4 weeks post-op. After this visit, the

study will perform blood tests which include a complete blood count, protime and comprehensive metabolic panel (CMP). These tests will be provided through a commercial testing laboratory (eg. Diagnostic Laboratory Services, Inc.). The results of these clinical laboratory test results will be compared to the pre-operative PET imaging findings to investigate whether PET may be predictive of post-surgery liver function. This is consistent with Specific Aim 3 of the research study.

If the research subject agrees, additional research blood samples will be obtained immediately before and 1-4 week following liver resection surgery. These samples will be sent to UHCC for analysis. If the patient is having other blood tests, these samples can be collected with the other tests. Using sterile technique, an intravenous needle will be inserted by a trained IV technician into an arm vein. A total of approximately 4 tsp of blood will be withdrawn into two vacutainer tubes. The sample centrifuged to isolate plasma fraction, buffy coat, and pellet. These samples will be frozen and analyzed in batches later to provide data on germline DNA, RNA, microRNA, and circulating RNA/DNA. The same procedure will be applied to the pre-surgery and post-surgery sample collections. If the results of a recent (within 3 months) fasting lipid profile (ie. cholesterol blood test) are not available, a fasting lipid profile will be obtained by Diagnostic Laboratory Services at the time other baseline laboratory samples are being obtained by the study. This information will be compared with the PET imaging and tissue expression data, providing reference values for the genomic analyses (Specific Aim 2). These blood samples will be used only to pursue the goals of this study, and will not be stored and made available to other research studies.

I.8. Clinical Procedures or other Measures to Monitor the Effects of the Drug in Human Subjects

Vital signs (e.g., temperature, heart rate, blood pressure, respiratory rate) will be measured on each subject prior to radiotracer administration. Vital signs will be repeated at the conclusion of PET imaging. During and after the PET/CT scanning procedure, subjects will be asked if they are experiencing any unusual symptoms.

I.9. Policy regarding human subjects and informed consent

Research involving human subjects is covered by federal law and institutional policies. All research conducted at our institution requires review and pre-approval by a Scientific Review Committee followed by approval from an Institutional Review Board. Subjects will be given a Queen's Medical Center IRB-approved consent.

I.10. PET/CT Protocol

Instrument: Gemini TF-64 PET/CT (Philips Medical Systems N.A., Bothell, WA)

Scan Protocol: Low-Dose CT, Liver PET Protocol

Isotope Dose: 18F-Fluoromethylcholine, **0.09 mCi/kg** patient body weight on day of scan.

1. A copy of the signed consent form will be faxed to the Research Regulatory Office.
 2. Height, sex, and age of the patient are recorded. Body weight, blood pressure, pulse, temperature and respiratory rate are obtained. The obtained weight is used to determine 18F-choline dose.
 3. Using sterile technique, an intravenous catheter is inserted by a trained IV technician into an arm vein. The catheters are flushed with sterile saline. The patient is placed in the supine position on the scanning table.
 4. A vial of 18F-choline containing a dose of 0.09 mCi/kg of body weight will be obtained from the cyclotron laboratory. A dose calibrator in the PET imaging suite will be used to confirm the actual administration dose. Only a dose falling within a 10% range of the calculated prescribed dose will be administered.
 5. A topographic scan will be performed to identify anatomical boundaries and the axial imaging field of view. The field of view is to encompass the liver, with 1cm margin from the upper limit of the field.
 6. A CT transmission scan in the supine position is acquired over the upper abdomen. The 64-channel helical CT scanning parameters were: 120 kV, 30-50 mA/slice, rotation time 0.75 sec, slice thickness/interval 5.0mm. No intravenous contrast will be used with CT.
 7. The 18F-choline is injected by bolus followed by a 10 cc saline flush. Image acquisition is begun immedi-
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ately with the flush in a dynamic mode using the following timing schema:

Frame #	Duration (seconds)
1-20	10
21-23	40
24-27	120
28-30	240
31	360

However, if it is determined in the initial 5 subjects that the dynamic imaging data fits well into a 1-tissue kinetic model of FCH uptake where activity levels eventually plateau or become linear, then the dynamic scan can be shortened (to 12-15 minutes, for example) and the dynamic frames curtailed accordingly.

8. A short 3-minute static scan of the liver will be performed subsequent to the dynamic scan, and rapidly reconstructed to provide the investigators an immediate image for review of quality control and biodistribution. The operating characteristics of this PET device are described elsewhere (Surti S, et al. *J Nucl Med.* 2007;48(3):471-480.)
9. After the scan, patient blood pressure, pulse, respiratory rate, and temperature are measured and recorded. Subjects are released pending investigator review of the vital signs and flow sheet.
10. Image reconstruction will employ a list-mode version of a maximum likelihood expectation maximization algorithm with a time-of-flight kernel applied in both the forward- and back-projection operations. CT data is used for attenuation correction. Reconstructed images in 3 orthogonal planes will be reviewed on a dedicated PET/CT viewing station and will be archived on removable media in DICOM format.

I.11. Facilities Data

The facilities used to carry out the activities described in this document are located at the Queen's Medical Center, 1301 Punchbowl St., Honolulu, HI 96818. These facilities include the Hamamatsu/Queen's PET Imaging Center which houses the tomograph, and the Liholiho Cyclotron Building which houses the accelerator. The accelerator used to produce radioisotopes is an 11 MeV RDS-111 (CTI, Knoxville, TN) with the required beam line and targetry systems. The radiochemistry laboratories house production systems and robotics, a pneumatic system for transfer of radiopharmaceutical to the imaging area, and adequate equipment and laboratory space for radiopharmaceutical production and quality control. The hot lab contains 2 hot cells (Von Gahlen, Netherlands), organic chemistry space for compound production and development, and a small radiopharmacy in the imaging suite. There is 110 square feet of radioanalytical instrumentation and desk space for radiochemistry work.

Handling of all radioisotopes is conducted under the supervision of the hospital Radiation Safety Office (RSO). RSO personnel will regularly monitor the radiopharmaceutical laboratories for radioisotope contamination. All personnel using radioisotopes will wear a film badge and those handling isotopes will wear finger ring badges to monitor integrated radiation absorbed dose. Disposal of radioactive waste is only in specially designated and properly shielded receptacles. The laboratory is monitored for radioactivity by external monitoring and wipe tests. The short half-life of fluorine-18 insures that no long-term radiation safety problem is engendered. All equipment is tested regularly as to its operation. Specifically, the dose calibrator is tested for constancy each day it is used, its linearity quarterly, and its accuracy annually. Logbooks record these measurements. Radiation survey instruments, including safety monitors (hand and foot) and personal dosimeters, are calibrated at least annually and following any repairs. All calibration procedures and schedules for radiation monitoring devices are reviewed and approved by the RSO. The PET imaging facilities are located in the first floor of Queen's Medical Center, adjacent to the Nuclear Medicine clinic. This area houses the PET/CT device. There are existing waiting rooms and patient examination facilities in the clinic and physician and nursing personnel are available on premises. The rooms of the PET suite are the 1) two tomograph rooms, one containing a Hamamatsu SHR-22000 PET system, and another containing a Philips Gemini TF PET/CT scanner with 64-slice CT, 2) a control room with a small radiopharmacy laboratory, 3) the administration and patient holding room, and the 4) a data analysis and display room located in the Nuclear Medicine clinic.

I.12. Institutional Review Board Data

This study with [¹⁸F] fluorocholeline requires review by the Queen's Medical Center Institutional Review Board. Our institutional review process is composed of two parts. A Scientific Review Committee (SRC) preliminarily reviews all new research proposals with the exception of retrospective studies. If passed by the SRC, an Institutional Review Board (IRB) will then conduct the initial and continuing review and approval of any project involving human subject research. The address of the Queen's Medical Center IRB is:

Research and Institutional Review Committee
Institutional Review Board
The Queen's Medical Center
1301 Punchbowl Street, University Tower Room 505
Honolulu, HI 96813

Appendix: Standard Operating Procedures for Collection of Tumor and Liver Tissue Specimens

1.0 Scope and Applicability

This standard operating procedure (SOP) describes the methods for tissue sample collection, preparation, storage, and reporting.

2.0 Definition

2.1 Research Subject Information and Consent Form: This is the signed document allowing consent (i.e., Chain of Custody records), by the patient and surgeon. This document must be available before or during the surgical procedure before the patient is included into his study. A Photo copy of the document must be available at the Queen's Medical Center, Hawaii Medical Center Pathology laboratory and the Cancer Research Center oh Hawai'I (CRCH).

2.2 Surplus Tissue: Tissue normally discarded by the pathology lab. This material will be designated by pathology personnel and retained by Cancer Research Center of Hawaii personnel.

3.0 Cautions

3.1 Tissue samples must be handled expediently and properly, as the tissue quality will be adversely affected by prolonged transport time or changes in temperature.

3.2 To minimize the potential for cross-contamination of samples, sterile technique and use of sterile equipment or sterilized instruments will be available through Cancer Research Center of Hawaii personnel.

1. Sterile gloves, Derm Assist, size 7.5, R.Weinstein #132750, 50 pairs per box
2. Sterile gloves, Triflex, size 7.5, Cardinal #2D7254, 40 pairs/box
3. Sterile gauze, ProAdvantage, 4 x 4, R.Weinstein #P157025, 25 x2 /package
4. Sterilized forceps, VWR, 6" round tip dissecting, 82027-378, pack of 12
5. Sterilized knife handle, Cardinal #4781, each.
6. Sterilized Autopsy Knife Blade, Cardinal, #4783, each.

4.0 Responsibilities

4.1 Surgical staff (Surgical Associates office 523-0166) is responsible for identifying potentially eligible cases and notifying CRCH lab (440-5238) of patient's surgical date and time.

4.2 CRCH is responsible for notifying the pathology laboratory (547-6536) of the surgery and preparing instrument sterilization and supplies. CRCH is also responsible for coordinating and monitoring tissue sample collection, transportation and providing technical assistance as needed.

4.3 Pathology Lab is responsible for receiving and analyzing the tissue samples, and aliquoting a portion of the desired entity to CRCH.

5.0 Apparatus and Materials

- 5.1 Gloves, sterile **
- 5.2 Gauze, sterile**
- 5.3 Forceps, dissecting, sterilized**
- 5.4 Knife handle for Autopsy knife blade, sterilized**
- 5.5 Knife blade, autopsy, disposable, sterilized**
- 5.6 Cooler, Igloo transport, with three (3) sterile specimen containers and ice*
- 5.7 Specimen ID Labels, preprinted*
- 5.8 Specimen Accession sheet*
- 5.9 Zip-lock Bags*
- 5.10 Surgical blades, sterile*
- 5.11 Cryogenic storage Boxes*
- 5.12 Allprotect, Qiagen, #76405 or RNA Later*
- 5.13 Formalin*

** Item will be used by hospital

. * Item will be used by CRCH.

6.0 Procedure

6.1 Sample Collection Criteria:

6.1.1. Any fresh surgical tissue from human liver samples that have been identified by surgeon.

6.1.2. Tissue selection based on (1) representative histology from primary site, (2) correspondence to a specific area on PET/CT if identified by radiologist, (3) minimal necrosis, (4) sufficient residual tissue to constitute Surplus tissue requirement. Biopsy samples should not be selected unless the pathology lab has approved its availability.

6.1.3. At minimum, two 0.5 cm cubes, one of tumor and one of normal tissue are requested.

6.2 Sample Preparation:

6.2.1 Surgical team will deliver fresh, specimen to Pathology lab.

6.2.2 Gloves must be worn when handling or cutting liver sections.

6.2.3 **Use a new, sterilized, disposable autopsy blade and forcep** to minimize the potential for RNA contamination of samples. Sections for CRCH should be taken using “Sterile Technique.”

6.2.4 At minimum, two core cubes are requested. For large tumors (> 5 cm greatest diam.), multiple tumor and normal specimens will be collected and numbered A1, A2 etc:

- **Section A for tumor** – Minimum 0.5 cm. cube. Label as “Sample _A”
- **Section B for normal, adjacent** – 0.5 cm cube. Label as “Sample _B”
- **Sections C or more** – 0.5 cm.cube. Label as “Samples C,” etc. May be substituted as necessary.
- **Section for non-liver** – No sections, unless directed by the P.I., surgeon, or pathologist.

6.2.5 All sections should be labeled and provided a discrete accession sequence under the CRCH numbering scheme. Record each sample description on the **Specimen Accession Form**, as approved by Pathology.

6.3 Documentation/Reporting:

6.3.1 CRCH will retrieve the subject’s tissue sample from HMC and process the tissue sample according to the SOP.

6.3.2 The specimen IDs are pre-printed and labels will be attached to the **Specimen Accession Form**. ID labels are meant to be placed on the sterile specimen container at the time of collection.

6.3.3 The CRCH number must be recorded on the signed **Research Subject Information and Consent Form**. Please review the form for the appropriate signatures. Incomplete forms will be rejected, and no tissue collection will be done.

6.3.4 Record the information requested on the **Specimen Accession Form**. Please write or Addressograph:

- 6.3.4.1 Patient name,
- 6.3.4.2 Physician,
- 6.3.4.3 Surgical procedure,
- 6.3.4.4 Surgery date,
- 6.3.4.5 Surgery time,
- 6.3.4.6 Patient Consent Form, yes or no,
- 6.3.4.7 Time delivered to pathology lab
- 6.3.4.8 Date and time of pick-up
- 6.3.4.9 Initials of person doing pick-up

If the pre- or post-operation diagnosis is available, please indicate them under “Relevant Information.” Please write legibly and provide remarks for each sample if needed with any issues or concerns.

6.3.5 **One sample per accession**(i.e., Do Not mark more than one site per entry.) The Specimen Accession Form must be filled.

6.3.6 A finalized copy of the surgical report is requested. This may be either faxed or mailed to:
Brenda Hernandez
1236 Lauhala street, Suite 407
Honolulu, Hawaii 96813
Fax: (808) 586-2982

6.4 Transporting Fresh tissue to CRCH:

6.4.1 Prepare ice chips or cool packs. Depending on the number of samples to be transported, the cool chest is hard sided and contains enough ice to cool tissues, but not freeze. Study staff should put the cool packs in a refrigerator or standard freezer. (**Note: Do not put the cool packs in the -80 C freezer**).

6.4.2 Put sterile specimen containers (6) in transport cool chest. Also, one zip-lock bag, gallon sized.

6.5 Handling and Storage.

6.5.1 Liver samples will be cut using a sterile technique into 4 equal parts:

6.5.1.1 RNA Later, stored in -20c

6.5.1.2 Allprotect, stored in -80c

6.5.1.3 Cryovial storage, stored in liquid nitrogen

6.5.1.4 Formalin fixation, followed within 24 hours by tissue processing. All excess tissue given will be processed, rather than discarded.

6.5.2 Sections will be accessioned onto a CRCH database and the exact locations of the different samples will be plotted.

7 Procedure

7.1 Surgical Associates (523-0166) will notify CRCH Lab (440-5238) of surgical scheduling of a prospective participant. This must include:

7.1.1 Patient identifier.

7.1.2 Surgical date.

7.1.3 Surgical time.

7.2 CRCH lab will notify pathology lab of the scheduling.

7.3 CRCH will arrange transportation and supplies for the surgical collection.

7.4 On the day of the surgery, surgery staff (547-6260) will notify CRCH within 30 minutes of expected dissection.

7.5 The pathology receives and dissects specimen. Instruments and tools will be handled by CRCH lab.

7.6 CRCH transports the tissue specimens back to CRCH

7.7 CRCH further dissects the specimen into the following components

7.7.1 DNA, RNA, and protein

7.7.1.1 Allprotect or RNA Later

7.7.2 Cryopreservation, Snap freezing into liquid nitrogen

7.7.3 Fresh frozen paraffin embedded (FFPE), permanent storage

8.0 Records

8.1 CRCH will secure and retain all pertinent records as required by HIPAA.

9.0 Quality Control and Quality Assurance

9.1 To ensure tissue sample/data quality, CRCH will monitor the tissue sample collection process. If sample quality or any processing issues occur, CRCH will modify the SOP and notify all offices and/or labs involved to resolve any problems or issues.

10.0 Reference:

N/A
