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Functional Liver Imaging with Sulfur Colloid SPECT/CT in Primary and Metastatic Liver Cancer Patients Receiving Liver-Directed Treatment: A Pilot Study

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TABLE OF CONTENTS

1.0 INTRODUCTION

Title	*Functional Liver Imaging with Sulfur Colloid SPECT/CT in Primary and Metastatic Liver Cancer Patients Receiving Liver-Directed Treatment: A Pilot Study*
Short Title	*Sulfur Colloid SPECT/CT for Liver Cancers*
Phase	*Pilot Study*
Methodology	*Single Arm Open-Label*
Study Duration	*4 years*
Study Center(s)	*University of Washington Medical Center*
Objectives	*Study the use of SPECT/CT imaging with ^{99m}Tc sulfur colloid to characterize functional liver in patients receiving liver-directed treatments for liver cancers, and characterize liver tissue response to these treatments. The long-term goal is to use functional liver imaging to personalize liver-directed therapies in liver cancer patients by improving risk stratification and reduce treatment-related hepatotoxicity.*
Number of Subjects	*60 evaluable subjects*
Diagnosis and Main Inclusion Criteria	*Patients diagnosed with primary (hepatocellular carcinoma, cholangiocarcinoma) and metastatic liver cancers receiving liver-directed therapies (external beam radiation therapy, surgery).*
Study Product, Dose, Route, Regimen	*^{99m}Tc sulfur colloid SPECT/CT scans*
Duration of administration	*Drug administration will consist of 3 SPECT/CT scans with ^{99m}Tc sulfur colloid (one baseline scan which is standard of care, two follow-up scans which are experimental and paid for by departmental research funds).*
Statistical Methodology	*^{99m}Tc sulfur colloid SPECT/CT parameters will be extracted, selected, and tested for statistical association to clinical liver function status and post-treatment toxicity using a variety of statistical methodologies. Receiver-operator characteristic (ROC) analysis will determine the optimal operating point for liver function classification accuracy using SC SPECT/CT parameters. Hypothesis testing of differences between independent groups will proceed using non-parametric Wilcoxon rank-sum between 2 groups or Kruskal-Wallis ANOVA between >2 groups. Hypothesis testing of differences between imaging time points of the same subjects will proceed using non-parametric Wilcoxon sign-rank or Friedman ANOVA paired comparison. Survival analysis will consist of Kaplan-Meier estimation, log rank testing, and Cox proportional hazard regression. Dose-response models will be constructed from linear and logistic regression. Prediction models will utilize robust feature selection and feature fitting through multi-fold cross validation as part of a general machine learning framework.*

This document is a protocol for a human research study. This study is to be conducted according to US and international regulations, applicable government regulations and Institutional research policies and procedures.

2.0 BACKGROUND

Study Disease

Hepatocellular carcinoma (HCC) is the most common primary liver cancer in the world and is one of the most rapidly growing causes of cancer-related death in the United States [1]. Multiple liver-directed treatment options are available to HCC patients, including liver transplantation, surgical resection,

radiofrequency ablation, catheter-based therapies, and radiation therapy (RT). HCC tumors often arise in the background of chronic liver disease such as alcohol, viral hepatitis, autoimmune hepatitis, or non-alcoholic fatty liver disease-related cirrhosis. Patients with cirrhosis commonly have impaired liver function, which plays a central role in determining which treatments patients can safely receive and how aggressive these treatments can be. Ultimately, tumor eradication with preservation of liver function is the desired treatment goal for HCC patients.

Technological advances in the field of radiation oncology have now made it possible to deliver high ablative or near-ablative doses of focal partial liver radiation with excellent local control rates of 70-90% after stereotactic body radiation therapy or proton beam therapy [2-4]. Similarly, advances in surgical techniques along with proper patient selection has allowed for encouraging 5-year overall survival rates of 50-70% despite more extensive resections [5]. Liver failure is the most serious and major cause of morbidity and mortality after treatment in HCC patients and arises primarily as a result of inadequate functional liver reserve post-treatment. Despite the most sophisticated radiation techniques, radiation-induced liver disease (RILD) can still develop in 5-67% of patients, depending on the baseline liver function and volume of liver irradiated, with fatal RILD in up to 5-7% [6-9]. Post-hepatectomy liver failure (PHLF) in resected HCC patients can occur in 5-20% of patients and is primarily dependent on the extent of resection and degree of pre-surgical liver dysfunction [10-12]. Potential reasons for the difficulties in mitigating these RILD and PHLF rates are the reliance on conventional assessments of liver function and on anatomical information of liver function to predict post-treatment liver toxicities. Furthermore, there are a group of patients that may not be offered potentially curative partial liver radiation or partial hepatectomy due to the fear of RILD or PHLF based on these crude conventional measurements of liver function and reserve post-treatment.

Currently, evaluation of global liver function routinely incorporates conventional laboratory blood tests (e.g. bilirubin, albumin, coagulation factors) and clinical variables (e.g. presence of ascites or hepatic encephalopathy) that indirectly measure liver function. They form the basis of clinical scoring systems such as the Child-Turcotte-Pugh (CTP) system that characterize liver dysfunction. However, these assessment tools are susceptible to subjectivity (clinical assessment of degree or severity of ascites and hepatic encephalopathy) and confounding factors (malnutrition affecting albumin) and may be unreliable in predicting hepatotoxicity after liver-directed treatment [13]. Small changes in the component lab values, for example, can lead to more significant score changes that may not truly reflect liver dysfunction, and transition between CTP classes can have major impact on patient management.

Perhaps more importantly, current models estimating the risk of liver toxicity are based upon estimating the threshold volume of non-tumor liver receiving a certain dose of radiation (RILD prediction) or the estimated volume of liver remaining after surgical resection (PHLF prediction) that is defined and delineated on computed tomography (CT) imaging. CT volumetry, however, is based solely on anatomic characteristics and provides no information on the spatial functional capacity or heterogeneity of the liver parenchyma. Furthermore, many patients planned to undergo RT or surgery have already received prior liver-directed or systemic therapies. It is known that the number of prior liver-directed therapies is a major contributor to worsening of liver function after RT [14], and preoperative chemotherapy can cause liver injury that results in increased morbidity and mortality after surgery [15]. It may be possible that the wide ranges in reported RILD and PHLF are due in part to the unaccounted effects of prior liver-directed and systemic therapies on liver function where regions of compromised liver function are considered “normal” when they are in fact dysfunctional. Thus, an unmet need for the treatment of HCC patients is the ability to accurately depict <u>global and regional</u> liver function in order to better risk stratify patients *a priori* and personalize treatments by preferentially avoiding regions of highly functioning liver. This unmet need also exists for certain patients with metastatic cancer to the liver. Many patients with colorectal cancer metastatic to the liver planned to receive surgical resection of their liver disease, for example, have received significant amount of systemic chemotherapy prior to surgery that results in steatohepatitis. Studies have shown that these patients are at high risk for perioperative morbidity and mortality [15]. Being able to more accurately assess the function of the future liver remnant noninvasively, therefore, would also be valuable.

Rationale for Use of SPECT/CT ^{99m}Tc-Sulfur Colloid Scans

^{99m}Tc sulfur colloid single-photon emission computerized tomography (SC SPECT/CT) imaging is a well-established FDA-approved diagnostic imaging modality for the evaluation of hepatic function, introduced by Harper et al. in 1965 [16]. Colloidal particles such as SC are primarily taken up and cleared by the reticuloendothelial (Kupffer) cells that line the sinusoids of the liver and serve as supporting cells to hepatocytes, as well as the spleen and bone marrow. It has been established that a close correlation exists between the function of hepatocytes and Kupffer cells. Hepatocytes and Kupffer cells also exhibit similar perfusion characteristics, are equally affected by liver fibrosis, and are proportionately distributed within the liver [17, 18]. Furthermore, multiple studies have demonstrated that SC distribution within the liver correlates well with histology in explanted livers [19], presence and severity of cirrhosis [20], and long-term clinical outcomes of hepatic failure-related mortality [21]. SC function, therefore, can be used as a surrogate for hepatic function.

While SC SPECT/CT is well-suited for the proposed aims, there are other imaging options. HIDA (^{99m}Tc-acetanilide iminodiacetic acid) or mebrofenin SPECT is directly related to hepatocyte function [22], but HIDA is preferentially extracted to the bile ducts, producing distinct uptake patterns which would confound the proposed heterogeneity analysis. Additionally, HIDA protocols use dynamic SPECT, which presents challenges such as increased imaging time and analysis workload, uncertain impact of detector geometric dependence over the dynamic imaging course, and general limitations of image-based input functions for SPECT [23]. MRI tracers are of interest including Gd-EOB-DTPA, a hepatocyte-specific contrast agent [24, 25], as well non-specific Gd contrast, the perfusion of which may correlate to ICG clearance [26]. While promising, MRI is more problematic for radiomics and correlation with radiation dose maps due to the possibility of geometric distortion, susceptibility artifacts from implanted fiducial markers, and issues with quantitative accuracy and uniformity inherent to MRI.

Clinical Data to Date

Detection of livery injury by radiation was first demonstrated in the late 1960's using 2-dimensional planar scintigraphy [27-29]. The inability to obtain 3-dimenstional spatial assessment of SC distribution in the liver and spleen hampered the routine use of SC in the clinic as well as application into the radiation and surgical oncology fields. Recently, the development of SPECT/CT technology has allowed improved regional contrast resolution relative to planar scintigraphy between functional, dysfunctional and non-functional liver from tomographic image acquisition, along with increased accuracy in local SC activity estimation from CT-based attenuation correction [30, 31]. Modern SC SPECT/CT imaging techniques, therefore, provide the potential for volumetric and spatial characterization of liver function prior to and in response to therapy. Preliminary retrospective data from our group have shown that measuring "total liver function" (derived from the product of functional liver volume and liver-to-spleen mean uptake) on pre-treatment SC SPECT/CT in 30 HCC patients may improve risk stratification and outcome prediction [32]. These data need to be validated prospectively with standardization of imaging protocols. Furthermore, changes in global or regional liver function as assessed with SC SPECT/CT, however, have not been studied in the context of RT and surgical resection of the liver.

Risks/Benefits

The primary risk to patients in this study is the risk associated with the two additional SPECT/CT ^{99m}Tc-SC scans. The radiation treatments or surgery that patients receive will be standard of care. Risks associated with the two SPECT/CT ^{99m}Tc-SC scans include: patient time (approximately 1 hour per scan); radiation dose from radioactive tracers and CT scans (up to ~ 30 mSv per session, which is about 0.1% of the usual radiation dose for liver cancer treatment, typically around 60 Gy), and intravenous injection and associated potential for bleeding, pain, infection, and injection site reaction.

In patients who opt to undergo the indocyanine green clearance study, there is the risk of an additional intravenous access procedure for the injection of the indocyanine green dye (injection of the indocyanine green must be separate from the intravenous access of the blood draw). Please see consent form for further details on potential risks.

The benefit gained from this study is the knowledge of whether SPECT/CT ^{99m}Tc-SC scans can identify, visualize, and quantify functional liver tissue. In doing so, we can preferentially protect those liver regions from radiation to decrease radiation induced liver injury in patients treated with radiation therapy. In surgical patients, we can potentially predict the risk of liver decompensation and peri-operative morbidity more accurately prior to surgery by more accurately quantifying the function of the future liver remnant. We will also learn about the effect of radiation and surgery on the spatial and temporal dynamics of liver function and recovery, which can help us improve and potentially expand our patient selection for aggressive liver-directed therapies and design individualized treatments to safely treat liver tumors while minimizing liver toxicity.

3.0 STUDY OBJECTIVES

3.1 Primary Objectives

a) Develop new quantitative metrics of *baseline* SC uptake on SPECT/CT imaging and correlate with clinical parameters of liver function and clinical outcomes in liver cancer patients receiving RT or surgery.

Hypothesis: Global metrics of baseline pre-treatment SC uptake on SPECT/CT imaging are more predictive for survival than conventional clinical measures of liver function and are associated with ICG clearance. For patients receiving surgery, the volume of the future liver remnant (FLR) as defined by SC SPECT/CT imaging (functional FLR) is more predictive for PHLF than CT-defined FLR volumes. Heterogeneity metrics of baseline SC uptake are associated with the severity of cirrhosis and prior liver-directed therapy; specifically, SC uptake within the liver is more heterogeneous in less compensated livers and in those that have received a higher number of liver-directed therapies.

b) Correlate *post-treatment* changes in SC uptake on SPECT/CT imaging with changes clinical liver function.

Hypothesis: Changes in SC uptake between pre-RT and mid-RT/post-RT SPECT/CT scans are associated with changes in clinical liver function in response to RT and are predictive of liver toxicity at earlier time points compared to clinical assessments of liver function. SC uptake in the FLR (non-treated) is stable and preserved over time between pre-surgery and immediately post-surgery (3-5 days postoperative) scans.

Secondary Objectives

a) Estimate the dose response relationship on multiple spatial scales (global liver, regional liver, liver image voxel) between radiation dose and changes in SC uptake, both acutely (mid-RT) and subacutely (1 month post-RT), using SC SPECT/CT imaging.

Hypothesis: Deficits in SC uptake due to absorbed dose is governed by a linear dose relationship. As the dose delivered to the region/voxel increases, the uptake in SC decreases in a linear fashion.

b) Estimate the degree of radiation response in liver tissue with varying levels of function (i.e. compare radiation dose response of well compensated livers against less compensated livers).

This may establish a threshold of liver tissue that would be "safe" to treat without significantly affecting liver function.

Hypothesis: Varying degrees of cirrhosis have different radiation dose response curves; specifically, more cirrhotic livers have a lower threshold for injury (more radiosensitive) than less cirrhotic or non-cirrhotic livers. The dose-response relationship can be modeled either as a linear or sigmoid function.

c) Correlate SC uptake on SPECT/CT imaging in FLR with extent of liver hypertrophy after surgical resection.

Hypothesis: In patients undergoing surgery, the functional FLR is predictive of the adequacy of the FLR and the extent of liver hypertrophy after hepatectomy. Higher functional FLR decreases the risk of PHLF and has a higher capacity and ability to hypertrophy compared to less functional FLR.

4.0 STUDY DESIGN

General Design

This is a pilot study of utilizing SPECT/CT imaging with ^{99m}Tc-SC to accurately identify and quantitate functional liver tissue on serial imaging in patients receiving RT or surgery for liver cancer. The long-term goals are to use functional imaging to improve risk stratification for selecting which patients should undergo aggressive local treatments with RT or surgery and to aid in RT planning by preferentially sparing functional liver tissue, thereby leading to reduced liver toxicity. There will be two cohorts of patients: those receiving RT (Cohort A) and those undergoing surgery (Cohort B). A total of 60 patients will be enrolled (Cohort A, n = 40; Cohort B, n = 20).

All patients will have a total of 3 SPECT/CT imaging with ^{99m}Tc-SC. The first scan in both cohorts will be routine medical care (not experimental) and will take place prior to initiation of RT or surgery. Two follow up scans will be part of the protocol. In cohort A, the first follow up scan will occur at mid-RT, and the second one about 1 month post-RT (prior to any potential anatomical liver changes). In cohort B, the first follow-up scan will occur 3-5 days postoperatively (prior to any potential liver hypertrophy), and the second one about 1month post-operatively (when liver hypertrophy is expected to occur).

Endpoints

4.1.1 Primary Endpoints

a) In this study, patients with liver cancer will undergo SPECT/CT imaging with ^{99m}Tc-SC at the time of RT planning for cohort A and within 1-2 weeks prior to surgery for cohort B. All image datasets will be imported into MIM 6.0, and fused with the RT planning CT and radiation plan for cohort A and with the contrast-enhanced diagnostic CT or MRI scan.

To characterize whole-organ liver function, all pre-treatment SPECT/CT images will be analyzed for 1) ratios of maximum, mean, and total liver SC uptake relative to spleen SC uptake (L/S) and 2) volumetric SC parameters such as the functional liver volume (FLV) formed by 58% maximum image intensity threshold segmentation. Optimal image thresholds for SC SPECT parameter association to CTP classification (A vs. B/C class) will be interrogated by receiver-operator characteristic (ROC) analysis. Image thresholds that yield maximum AUC in quantitative parameters such as FLV ratio, L/S ratio, and their total liver function (TLF) product will be propagated to the remaining analyses. In cohort A receiving RT, planned dose-volume and dose-function metrics within the tumor-subtracted liver and functional liver volume(s) will

feature mean dose, $D_{xx\%}$ (the dose given to xx% of liver volume), V_{zz} (the liver volume which receives zz Gy), and fV_{zz} (the percentage of total liver function which receives zz Gy).

To characterize spatial distribution of functional liver, radiomics will be applied to SC SPECT/CT images via a locally-developed open source radiomics toolkit [33]. In addition to the whole-organ SC SPECT/CT metrics descried above, radiomic features will include intensity histogram features, along with a variety of textural features derived from co-occurrence matrices, neighborhood difference matrices, and zone size matrices [34-36].

As a validation step, these baseline SC imaging data will be correlated to indocyanine green (ICG) clearance in patients (in those who opt to undergo this study), which assesses liver function based on the rate of clearance of a contrast agent over serial blood draws, and is considered a standard assessment of global liver function. ICG is a water-soluble compound that is selectively taken up by hepatocytes and transported into the biliary system without undergoing metabolism [37]. ICG clearance, therefore, can be utilized as an index of functioning hepatocyte mass [38] and is correlated with prediction of liver dysfunction and mortality associated with surgical resection [39]. The retention rate of ICG 15 minutes after administration (ICG-R15), a ratio of the ICG concentration at 15 minutes to its initial concentration in the blood, will be used to benchmark SC uptake metrics against. Higher ICG-R15 rates indicate worse liver function and would be expected to be correlated with lower global SC uptake metrics.

Functional FLR will be generated by calculating the volume of liver within the optimal image threshold as defined above. Anatomic FLR will be delineated and calculated on CT or MRI images using portal and hepatic veins as landmarks for segmental division. Logistic regression analyses and Pearson chi-square test will be used to compare the ability to predict for PHLR between functional FLR and anatomic FLR.

b) Correlate *post-treatment* changes in SC uptake on SPECT/CT imaging with changes clinical liver function. Relative changes in the parameters identified at baseline will be tested for correlation to changes in clinical liver function, including CTP score and ALBI grade, as well as their individual constituents.

4.1.2 Secondary Endpoints

Patients will receive two follow-up SC SPECT/CT scans: mid-radiation treatment (cohort A) or 3-5 days post-surgery (cohort B) and 1 month (cohort A) or 6 months (cohort B). For the second aims (a) and (b) looking at liver tissue inside the radiation field, the images will be processed similarly to the primary endpoints. Regional functional liver changes from scatter, collimator, and attenuation-corrected SPECT/CT images will be modeled as a function of regional radiation dose. Three types of dose-response models will be defined to predict changes in liver function status:

Dichotomous dose-response (complete function loss): Single dose thresholds on co-registered baseline and post-treatment SPECT/CT will be applied to investigate a binary threshold for functional loss. Image voxels exposed to a radiation dose above the selected threshold will be excluded when calculating the percent change in function liver image parameters. The product of functional liver remnant following complete loss of function on imaging (post-to-pre-treatment ratio) and baseline liver function status (e.g. ICG clearance, bilirubin, etc.) will be compared to the repeat functional liver status measured post-RT.

Binned dose-response (partial function loss): Dose threshold bins will be co-registered to the baseline and post-treatment SPECT/CT in order to explore different thresholds of radiation leading to partial loss in lung function. For imaging voxels in each dose bin, the corresponding SC SPECT/CT uptake decline will be calculated following a count normalization outside the radiation field. Model-predicted change in

SC SPECT/CT image parameters will be compared against the measured change post-therapy. The product of functional liver remnant following partial loss of function on imaging (post-to-pre-treatment ratio) and baseline liver function status (e.g. ICG clearance, bilirubin, etc.) will be compared to the repeat functional liver status measured post-RT.

Continuous dose-response (function loss distribution): The continuous dose distribution will be co-registered to the baseline and post-treatment SPECT/CT. The relationship between dose and SC SPECT/CT uptake reduction at the voxel scale will be directly characterized via linear and logistic regression methods with noise suppressing fitting techniques (multiscale lung → lobar → voxel model). Goodness of model fits (pseudo R^2) using rigid and deformable registration of SPECT/CT images and RT dose will be compared. The product of functional liver remnant from the function loss distribution on imaging (post-to-pre-treatment ratio) and baseline liver function status (e.g. ICG clearance, bilirubin, etc.) will be compared to the repeat functional liver status measured post-RT.

Statistical Analysis

4.2.1 Primary Endpoints

a) Significant differences in Wilcoxon rank sum of ROC-optimized SC SPECT parameters will be tested between CTP classes. Spearman rank correlations between optimal SC SPECT parameters and clinical liver function parameters will be tabulated for the following categories: (1) composite liver function scoring systems (CTP score, ALBI grade), (2) individual quantitative liver function components (albumin, bilirubin, INR), and (3) individual qualitative clinical liver function components (encephalopathy, portal hypertension, ascites, and splenomegaly). Qualitative components will be treated as binary categorical variables (yes/no). Imaging and clinical parameters of liver function will be tested for association with overall survival. Continuous variables will be dichotomized above and below a threshold value that maximized accuracy of classification under ROC with balanced sensitivity and specificity. Univariate Cox proportional hazard regression will then be performed on all continuous and categorical variables. Statistically significant variables will serve as inputs into multivariate Cox proportional hazard regression. A stepwise backwards variable selection procedure will be followed to remove non-significant variables with high cross-correlation until the model log-likelihood is maximized.

   Because radiomics generates feature sets of ~50 metrics, machine learning methods will be utilized to develop predictive models that are robust against overfitting, utilizing feature selection and classification/regression methods such as support vector machines, Bayesian analysis, and decision trees. These machine learning methods will be compared against standard statistical methods, such as ROC analysis and Kaplan-Meier estimation / Cox regression, for association to clinical liver function, prior liver-directed therapies, clinical outcomes (i.e. survival), and toxicity (i.e. CTP score progression).

b) The same set of statistical analysis will be performed for association of post-treatment changes in SC uptake on SPECT/CT imaging to clinical endpoints. Machine learning methods will test whether post-treatment changes in SC SPECT/CT provide independently predictive information after adjusting for baseline parameters.

5.0 SUBJECT SELECTION

Inclusion Criteria

5.1.1 Patients with a diagnosis of HCC, intrahepatic cholangiocarcinoma, or metastatic liver cancer planned to receive definitive doses of radiation or surgical resection are eligible

5.1.2 Measurable hepatic disease and/or presence of vascular tumor thrombosis.

5.1.3 Diagnostic CT or MRI scan within 2 months of study entry.

5.1.4 There are no limits on prior therapy. Patients are allowed to have prior systemic therapy, radiation therapy, radiofrequency ablation, catheter-based therapies, and surgery. Patients are allowed to have concurrent chemotherapy with radiation treatment.

5.1.5 Patients >18 years old.

5.1.6 Ability to understand and the willingness to sign a written informed consent document.

Exclusion Criteria

5.1.7 Patients unable to tolerate a SPECT/CT ^{99m}Tc-SC scan.

5.1.8 Patients who are not planning to adhere to the required follow up schedule as outlined in this protocol.

5.1.9 Pregnant women.

5.1.10 Women of childbearing potential and men who are sexually active and not willing/able to use medically acceptable forms of contraception.

5.1.11 Patients unable to provide informed consent.

Recruitment

Subjects will be recruited from investigator clinical practices and include men and women with liver cancer who will be receiving definitive doses of radiation or surgical resection. Subjects will undergo an informed consent process in accordance with GCP (see section 11 Ethical Considerations). Informed consent will be obtained prior to the performance of any screening procedures.

6.0 SUBJECT REGISTRATION

Subjects will be registered by the FHCRC/UW Study Coordinator and entered into the Protocol Accrual Tracking System (PATS). Information regarding the PATS system is available at http://www.cancerconsortiumorg/rto/protocol_office/pats/. A complete, signed, study consent and HIPAA consent are required for registration.

7.0 TREATMENT PLAN

All patients will receive radiation or surgical treatment per standard of care. As part of this study, all patients will undergo three SC SPECT/CT scans (**Fig. 1**):

1) First scan will prior to the start of treatment. This scan will be standard of care clinically for patients undergoing liver-directed therapy and will be at the time of radiation treatment planning in the treatment position for the RT patients (Cohort A) or within 2 weeks prior to surgical intervention for surgery patients (Cohort B).

2) Second scan will be mid-radiation treatment in Cohort A, to assess the acute response of functional liver to radiation. For patients treated with a 5 fraction regimen, this mid-treatment scan will occur after the 3rd fraction (+/- 2 days acceptable) and after the 8th fraction for patients treated with a 15 fraction regimen (+/- 2 days acceptable). For Cohort B, this scan will be within 3-5 days post-surgery to assess for the functional stability of the liver remnant.
3) Third scan will assess the longer term response of functional liver tissue to treatments. In Cohort A, this will be 1 month post-radiation (up to 2 months is acceptable). In Cohort B, this will be 1 month +/- 2 to 6 weeks post-surgery.

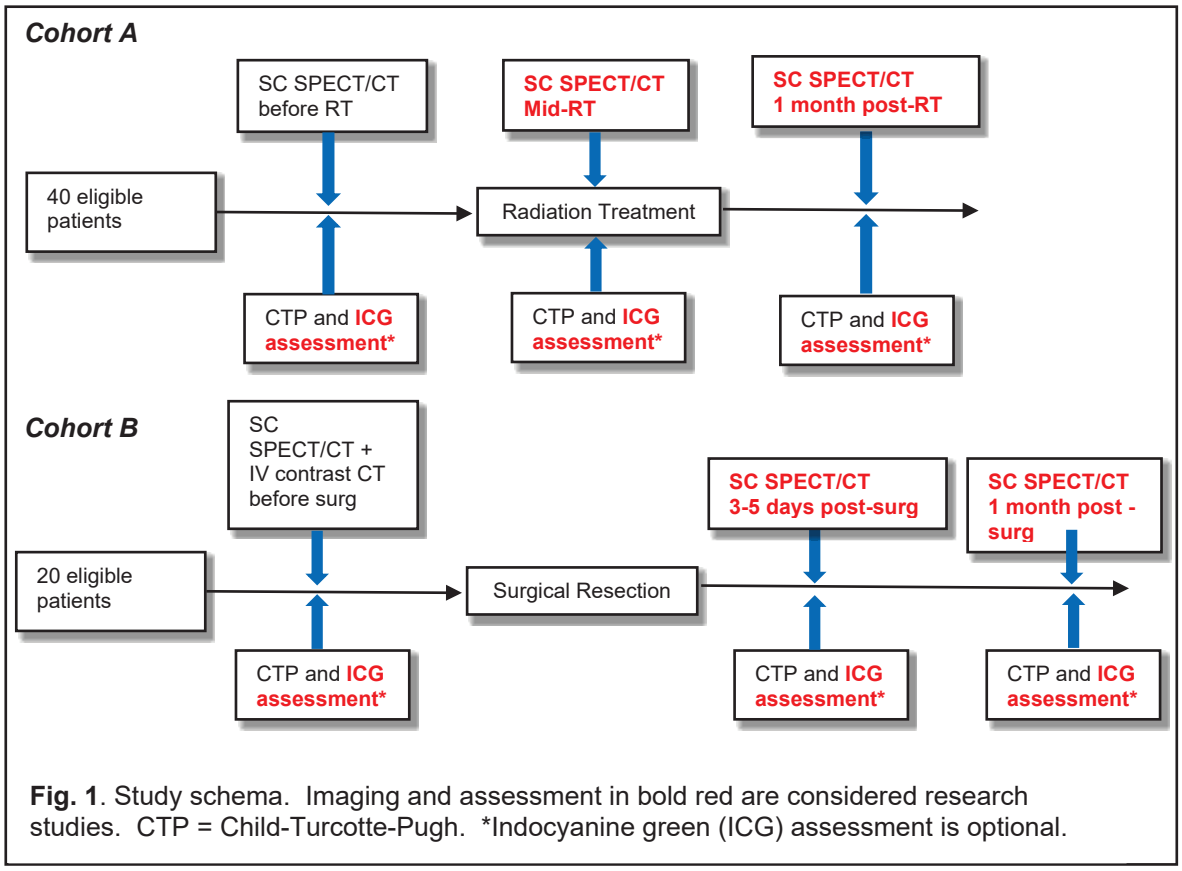

Fig. 1. Study schema. Imaging and assessment in bold red are considered research studies. CTP = Child-Turcotte-Pugh. *Indocyanine green (ICG) assessment is optional.

Follow-up of all patients will occur via standard clinical follow-up procedures by the treating physician. This clinical follow-up will include clinical exam, standard serum liver function tests and tumor markers, and CT or MRI scans for continued cancer surveillance and treatment response. Research staff will review clinical records at 6 months post-treatment. See Table 1 below for summary.

Table 1. Study Calendar.

Cohort A (Radiation Therapy Patients)

	Screening	Prior to Start of Radiation Treatment	Mid-Radiation Treatment	1-Month Post-Radiation Treatment
Informed Consent	X*			
Physical Exam	X			X
Medical History	X			X
Standard Serum Liver Function and Tumor Marker Tests†		X	X	X

Serum Pregnancy Test††	X			
ICG Clearance Test (optional)†		X*	X*	X*
CT or MRI scan‖	X			X
^{99m}Tc-SC SPECT/CT scan		X¶	X*	X*

*Denotes a research procedure; all others are standard of care.
†On the same day as ^{99m}Tc-SC SPECT/CT scans +/- 2 days.
**Complete blood count, complete metabolic panel, prothrombin time, partial thromboplastin time, AFP (for hepatocellular carcinomas), CA19-9 (for cholangiocarcinomas or metastatic pancreatic cancer), CEA (for metastatic colon cancer), CA-125 (for metastatic ovarian cancer).For patients receiving SBRT, patients will have blood drawn after fraction 3 +/- 1 fraction. For patients receiving proton beam therapy patients will have blood drawn every 7 days +/- 3 days
††For women of child bearing potential only.
‖Type of scan is up to the discretion of treating physician.
¶At the time of simulation for RT planning.

Cohort B (Surgery Patients)

	Screening	**Prior to Surgery**	**3-5 Days Post-Surgery (inpatient)**	**1 Month Post-Surgery +/- 2 to 6 weeks (outpatient)**
Informed Consent	X*			
Physical Exam	X			X
Medical History	X			X
Standard Serum Liver Function and Tumor Marker Tests†**		X	X	X
Serum Pregnancy Test††	X			
ICG Clearance Test†		X*	X*	X*
CT or MRI scan‖	X			X
^{99m}Tc-SC SPECT/CT scan + IV contrast enhanced abdominal CT scan		X¶	X*	X*

*Denotes a research procedure; all others are standard of care.
†On the same day as ^{99m}Tc-SC SPECT/CT scans +/- 2 days.
**Complete blood count, complete metabolic panel, prothrombin time, partial thromboplastin time, AFP (for hepatocellular carcinomas), CA19-9 (for cholangiocarcinomas or metastatic pancreatic cancer), CEA (for metastatic colon cancer), CA-125 (for metastatic ovarian cancer).
††For women of child bearing potential only.
‖Type of scan is up to the discretion of treating physician.
¶Within 2 weeks prior to surgery.

Imaging Protocol

SPECT/CT with ^{99m}Tc-SC will be performed per standard protocol, which is currently in routine clinical use. The protocol will consist of a SPECT/CT image acquisition on a dual head gamma camera and 16 slice CT scanner. Following the injection of 7 mCi (259 MBq) [99mTc] sulfur colloid, SPECT scans will be acquired 15 minutes post-injection over a fixed time-averaged frame (64 views, 20 sec/view, 180 degree arc). Emission images will be corrected for scatter, collimator-detector response, and attenuation using a tidal breathing end-exhale position CT image. Reconstructions will be performed with a variant of ordered subset expectation-maximization (OSEM) iterative algorithm. An initial baseline scan prior to RT or surgery will identify areas of functional liver. In the radiation therapy cohort, this scan will be in the radiation treatment position which can be fused precisely with the

radiation treatment planning scan, and will be used to identify functional liver tissue both inside and outside the radiation fields. Two follow-up scans will be used to determine the effects of RT and surgery on liver function. In the radiation therapy cohort, the first follow-up scan will be obtained mid-treatment and the second will be obtained 1 month post-treatment. Detailed radiation dose response curves will be generated from these data. In the surgery cohort, the first follow-up scan will be obtained 3-5 days post-surgery and the second will be obtained 1 month post-surgery +/- 2 to 6 weeks post-surgery.

For the surgery cohort, an additional IV contrast enhanced CT scan (70 second delay) will be obtained immediately following the SPECT/CT scan for all 3 SPECT/CT scans. Since the anatomic FLR will be delineated and calculated using portal and hepatic veins as landmarks for segmental division, these IV contrast CT scans will be important in identifying these blood vessels accurately.

Criteria for Removal/Withdrawal from Treatment

Patients will be withdrawn from treatment if their clinical conditions decline to the point that they are no longer able to tolerate SPECT/CT with ^{99m}Tc sulfur colloid. They will be withdrawn from treatment if they are enrolled into hospice.

Patients will still receive follow-up care per standard of care even if they withdraw from the study. If a subject withdraws consent to participate in the study or aspects of the study, attempts will be made to obtain permission to record at least survival data up to 6 months post-treatment.

8.0 DATA AND SAFETY MONITORING PLAN

Oversight for this study at UWMC will be provided by the Principal Investigator with delegation of appropriate responsibilities to sub-investigators and designated study personnel. They will ensure all entry criteria are met prior to the initiation of the protocol and all study procedures and reporting of adverse events are performed according to the IRB-approved protocol.

Institutional support of trial monitoring will be in accordance with the Fred Hutch/University of Washington Cancer Consortium Institutional Data and Safety Monitoring Plan. Under the provisions of this plan, Fred Hutch Clinical Research Support coordinates data and compliance monitoring conducted by consultants, contract research organizations, or Fred Hutch employees unaffiliated with the conduct of the study. Independent monitoring visits occur at specified intervals determined by the assessed risk level of the study and the findings of previous visits per the institutional DSMP.

In addition, protocols are reviewed at least annually and as needed by the Consortium Data and Safety Monitoring Committee (DSMC) and the Fred Hutch/University of Washington Cancer Consortium Institutional Review Board (IRB). The review committees evaluate accrual, adverse events, stopping rules, and adherence to the applicable data and safety monitoring plan for studies actively enrolling or treating patients. The IRB reviews the study progress and safety information to assess continued acceptability of the risk-benefit ratio for human subjects. Approval of committees as applicable is necessary to continue the study.

The trial will comply with the standard guidelines set forth by these regulatory committees and other institutional, state and federal guidelines.

9.0 ADVERSE EVENTS

9.1 Adverse Event (AE)

According to ICH guidelines (Federal Register. 1997; 62(90):25691-25709) and 21 CFR 312.32, IND Safety Reports, and ICH *E2A, Definitions and Standards for Expedited Reporting, an adverse event is defined as follows:*

An *adverse event* is any untoward medical occurrence in a clinical investigation subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Abnormal laboratory values for laboratory parameters specified in the study should not be recorded as an AE unless an intervention is required (repeat testing to confirm the abnormality is not considered intervention), the laboratory abnormality results in a serious adverse event or the adverse event results in study termination or interruption/discontinuation of study treatment.

Medical conditions present at screening (i.e., before the study treatment is administered) are not AEs and should not be recorded on adverse event pages of the CRFs. These medical conditions should be adequately documented on the subject chart. However, medical conditions present at baseline that worsen in intensity or frequency during the treatment or post-treatment periods should be reported and recorded as AEs.

9.2 Serious Adverse Event (SAE)

An adverse event should be classified as an SAE if it meets one of the following criteria:

Fatal:	AE results in death.
Life threatening:	The AEs placed the subject at immediate risk of death. This classification did not apply to an adverse event that hypothetically might cause death if it were more severe.
Hospitalization:	It required or prolonged inpatient hospitalization. Hospitalizations for elective medical or surgical procedures or treatments planned before enrollment in the treatment plan or routine check-ups are not SAEs by this criterion. Admission to a palliative unit or hospice care facility is not considered to be a hospitalization.
Disabling/incapacitating:	Resulted in a substantial and permanent disruption of the subject's ability to carry out normal life functions.
Congenital anomaly or birth defect:	An adverse outcome in a child or fetus of a subject exposed to the molecule or treatment plan regimen before conception or during pregnancy.
Medically significant:	The AE did not meet any of the above criteria, but could have jeopardized the subject and might have required medical or surgical intervention to prevent one of the outcomes listed above.

9.2 Unexpected Adverse Event

An unexpected AE is defined as an event that has a nature or severity, or frequency that is not consistent with the applicable investigator brochure, or the prior medical condition of the subject or other treatment given to the subject. "Unexpected," as used in this definition, refers to an adverse drug experience that has not been previously observed and reported in preclinical or clinical studies rather than an experience that has not been anticipated based on the pharmacological properties of the study drug.

9.3 Monitoring and Recordings Adverse Events

All AEs will be assessed by the investigator or qualified designee and recorded in the CRFs. The investigator should attempt to establish a diagnosis of the event on the basis of signs, symptoms and/or other clinical information. In such cases, the diagnosis should be documented as the AE and/or SAE and not described as the individual signs or symptoms. The following information should be recorded:

- Description of the AE using concise medical terminology
- Description as to whether or not the AE is serious, noting all criteria that apply
- The start date (date of AE onset)
- The stop date (date of AE resolution)
- The severity (grade) of the AE
- A description of the potential relatedness of the AE to the study procedure or other causality
- The action taken due to the AE
- The outcome of the AE

Subjects will be followed for safety per standard of care if subject terminates early or who experience a non-serious AE considered to be possibly or definitely related to study treatment.

9.4 Grading Adverse Event Severity

All AEs will be graded in severity according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. If a CTCAE criterion does not exist, the investigator should use the grade or adjectives: Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe), Grade 4 (life-threatening), or Grade 5 (fatal) to describe the maximum intensity of the AE.

9.5 Attribution of an Adverse Event

Association or relatedness to the study assessments will be assessed by the investigator as follows:

- Definite: The event follows a reasonable temporal sequence from exposure to the investigational agent, has been previously described in association with the investigational agent, and cannot reasonably be attributed to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications; AND the event disappears or improves with withdrawal of the investigational agent and/or re-appears on re-exposure.
- Probable: The event follows a reasonable temporal sequence from exposure to the investigational agent and has been previously been described in association with the investigational agent OR cannot reasonably be attributed to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.
- Possible: The event follows a reasonable temporal sequence from exposure to the investigational agent, but could be attributable to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.
- Unlikely: Toxicity is doubtfully related to the investigational agent(s). The event may be attributable to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.
- Unrelated: The event is clearly related to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.

For general AE assessment, an AE is considered related if it is assessed as definitely, probably, or possibly related; unrelated if it is assessed as unlikely related or unrelated.

9.6 Adverse Event Recording Period

AEs will be monitored and recorded in study-specific case report forms (CRFs) from the time of first research optional blood draw or imaging exposure on this study. AEs with an onset date prior to the first exposure to an investigational product will not be recorded, except in the case of clinically significant worsening of the AE during the specified AE monitoring time frame.

9.7 Adverse Event Reporting Requirements

The investigator or designee must report events to the FHCRC IRB in accordance with the policies of the IRB.

9.1 DATA MANAGEMENT/CONFIDENTIALITY

The investigator will ensure that data collected conform to all established guidelines. Each subject is assigned a unique patient number to assure subject confidentiality. Subjects will not be referred to by this number, by name, or by any other individual identifier in any publication or external presentation. The licensed medical records department, affiliated with the institution where the subject receives medical care, maintains all original inpatient and outpatient chart documents.

Subject research files are stored in a secure place (or database). Access is restricted to authorized personnel.

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