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Optimizing Outcomes of Patients with Advanced HCC Undergoing Immunotherapy Through Novel ^{68}Ga PSMA PET Imaging

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Optimizing outcomes of patients with advanced HCC undergoing immunotherapy through novel ^{68}Ga PSMA PET imaging

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LIST OF ABBREVIATIONS

AFP	Alpha-fetoprotein
Anti-VEGF	Anti–vascular endothelial growth factor
Anti-PD-L1	Anti-Programmed Death Ligand-1
CBC	Complete Blood Count
CI	Confidence Interval
CLMM	Cumulative Link Mixed Model
CMP	Comprehensive Metabolic Panel
CT	Computed Tomography
DCP	Des-gamma-carboxy Prothrombin
EDTA	Ethylenediaminetetraacetic acid
FDA	Food and Drug Administration
FDG	Fluorodeoxyglucose
^{68}Ga	68 Gallium
H&E	Hematoxylin and Eosin
HBN	Hepatobiliary Neoplasia
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HIPAA	Health Insurance Portability and Accountability Act
IDE	Investigational Device Exemption
IHC	Immunohistochemical
INR	International Normalized Ratio
IRB	Institutional Review Board
LI-RADS	Liver Imaging Reporting and Data System
MCR	Mayo Clinic Rochester
mRECIST	Modified Response Evaluation Criteria in Solid Tumors
MRI	Magnetic resonance imaging
MTV	Metabolic Tumor Volume
NGS	Next-Generation Sequencing
OS	Overall Survival
PET	Positron Emission Tomography
PFS	Progression-free Survival
PHI	Protected Health Information
PI	Principal Investigator
PRC	Pathology Research Core
PSMA	Prostate Specific Membrane Antigen
SOC	Standard of Care
SUVmax	Maximum Standardized Uptake Value

TOF	Time of Flight
UADE	Unanticipated Adverse Device Effect

Study Summary

Title	Optimizing outcomes of patients with advanced HCC undergoing immunotherapy through novel ^{68}Ga PSMA PET imaging
Running Title	Optimizing outcomes of patients with advanced HCC undergoing immunotherapy through novel ^{68}Ga PSMA PET imaging
IRB Protocol Number	21-007799
Phase	Phase II
Methodology	Prospective study
Overall Study Duration	Study will reach completion 3-years from the time the study opens to accrual
Patient Participation Duration	Patients will undergo up to 5 PET/CT scans or until disease progression whichever comes first.
Objectives	1. To test the performance of novel biomarkers derived from PSMA PET/CT to measure response compared to RECIST criteria, in advanced HCC patients treated with immunotherapy. 2. To assess the performance of novel biomarkers derived from PSMA PET/CT in predicting PFS in advanced HCC.
Number of Patients	30
Diagnosis and Main Inclusion Criteria	Adult patient with pathologically confirmed HCC not amenable to curative resection, transplantation or ablative therapies, who have radiographically measurable disease by RECIST and eligible for atezolizumab/bevacizumab front line therapy.
Duration of each study	2.5-3.5 hours
Statistical Methodology	We assume that 20 PSMA PET/CT positive patients will be identified by screening 30patients. We further assumed that patients with negative PSMA would have PFS at 6 months (PFS6) of 55% (historical data)[1]. Given 20 patients, we can detect a PFS6 difference of 26% (i.e. PFS6 drops from 55% to 29%) with 80% power assuming a one-sided p-value of 0.1 (using exact method). Aim 1: Time to response, per RECIST 1.1 and per PSMA PET/CT, will be correlated to each other using Cox proportional hazard model with response

	per RECIST 1.1 as the dependent varible and the time to response per PSMA PET/CT will be included in the model as a time-vaying covariate. Aim 2: PFS rate at 6 months will be calculated among PSMA PET/CT negative patients using Kaplan-Meier method with corresponding confidence interval calculated using Greenwood's formula.

1 Introduction

This document is a protocol for a human research study. This study will be carried out in accordance with the procedures described in this protocol, applicable United States government regulations, and Mayo Clinic policies and procedures.

1.1 *Background*

Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer. It is the sixth most common cancer worldwide and the fourth leading cause of cancer deaths.[2] Its incidence is rising faster than any other cancer in the United States[3] and is anticipated to continue to increase due to rising numbers of hepatitis C virus associated cirrhosis, particularly baby boomers with peak HCV cohort (1945-1965) and rising prevalence of non-alcoholic fatty liver disease.[4] Patients often present with advanced disease when first diagnosed. Even with recent advances in systemic treatments, median overall survival (OS) ranges from a dismal 10.7 to 13.6 months.[5, 6] Since 2007, sorafenib, an oral multikinase inhibitor, has been the first line systemic therapy for patients with advanced HCC. Recently, atezolizumab, an anti-PD-L1 antibody, in combination with bevacizumab, a monoclonal anti-VEGF antibody, showed superior outcomes (67.2% OS at 12 months vs. 54.6% with sorafenib)[1], which led to FDA approval of the combination as the first-line systemic treatment for advanced HCC. In contrast to clinical outcomes data, the combination showed a confirmed response rate of only 27.3% by conventional RECIST 1.1 and 33.2% by mRECIST criteria. These data highlight the significant unmet need for novel biomarkers of treatment response assessment in patients with HCC. In fact, in our current clinical practice at Mayo Clinic, all patients with advanced HCC continue to receive the combination until there are treatment-limiting toxicities or progression on imaging. National and international guidelines also do not have recommendations on specific response assessment criteria to use in the setting of novel immunotherapy.[7, 8] Thus, lack of validated noninvasive biomarkers are a critical barrier in individualizing therapeutic decisions in HCC patients treated with immunotherapy.

Gallium-68 (^{68}Ga) - Prostate Specific Membrane Antigen (PSMA)- N,N'-bis [2-hydroxy-5-(carboxyethyl)benzyl] ethylenediamine-N,N'-diacetic acid (HBED-CC) (also called PSMA-11), is a zinc metalloenzyme and a transmembrane protein that has a large extracellular domain, a transmembrane domain, and a short intracellular domain.[9] PSMA is overexpressed in prostate cancer cells. Therefore, PSMA is being evaluated worldwide including at our institution as a theranostic, i.e. combined diagnostic and therapeutic, target for prostate cancer.[10] Recent studies suggest that PSMA is not specific to prostate cancer but can be expressed in other solid tumors including sarcomas, thyroid and lung cancers.[11, 12] Two recent immunohistochemistry (IHC) studies suggest that HCC is one of the solid tumors that has higher-than-average expression of PSMA.[9, 13] In one study, 76 of 103 (74%) HCC lesions expressed PSMA – 27 lesions showed PSMA expression in >50% of tumor-associated vasculature and 49 lesions showed PSMA expression in

</=50% of tumor-associated vasculature.[9] In addition, high PSMA expression was associated with shorter overall survival. In another study, 79% of HCC lesions had moderate-to-high levels of PSMA expression.[13]

More recently, our group performed PSMA IHC on 148 HCCs from surgical resection or liver explant specimens. 90% of HCCs showed PSMA immunostaining which was localized to the tumor endothelium. More than 50% of HCCs showed 31%-100% PSMA immunostaining by area. In all cases the intensity of staining was strong with a score of 3 on a scale of 1 to 3. Two small imaging studies[14, 15] and a few anecdotal reports[16, 17] suggest that expression of PSMA in HCC can be translated to clinical imaging and can be detected non-invasively on PSMA PET. For instance, a pilot study compared 68Ga-PSMA PET/CT with 18F- FDG PET/CT in 7 patients with 41 HCC lesions, and correlated it to contrast enhancement on CT or MRI.[14] All but a single HCC lesion (n=36/37) were PSMA-avid with mean PSMA uptake in tumors being 3.6 times higher than background liver PSMA uptake. In contrast, only 10 lesions were FDG–avid. In addition, PSMA PET identified unexpected metastatic lesions in bone marrow, adrenal gland, and abdominal implant. Increased PSMA uptake in HCC on PET correlated with PSMA staining of endothelial cell lining of tumor neo-vasculature.

Our experience in Mayo Clinic with PSMA PET imaging of patients with HCC who are treated either with surgery, transplant or loco-regional modalities also supports the cited empirical data.

1.1.1 Anticipated Risks

CT technologies have been used in clinical applications for over 30 years and have a well-documented safety profile. Similarly, PET scanners are widely used in clinical practice. The PET/CT scanners do not involve any risks other than those normally associated with routine clinical PET or CT scans, which are performed in millions of patients in many hospitals all over the world. The following are some discomfort one may experience during a PET/CT scan session:

- Due to CT scanner: claustrophobia & anxiety. The amount of radiation received from the low dose non-contrast CT scan has a low risk of harmful effects.
- Due to intravenous access placement: Placing an IV for the radiotracer infusion may cause pain from the needle stick, bleeding, bruising, lightheadedness, or, on rare occasions, infection.
- Due to radiopharmaceutical ^{68}Ga-PSMA: Small risk of an allergic reaction. The amount of radiation received from the ^{68}Ga-PMSA-11 scan has a low risk of harmful effects.

PSMA uptake in non-LIRADS® 5 or biopsy proven lesions:
It is possible there may be detection of PSMA uptake in i) previously undetected, non-LI-RADS 5 intrahepatic observations or ii) PSMA uptake without a corresponding morphologic observation on CT or iii) extrahepatic PSMA uptake. Because we do not yet know the diagnostic accuracy of PSMA in patients with hepatocellular carcinoma, PSMA uptake found at the research PET or PET/CT outside of the known LI-RADS 5 lesion(s) and/or biopsy proven HCC will be disclosed to the referring provider for consideration of further evaluation. If the findings are not confirmed on follow-up (or prior) imaging or biopsy, then the research PET/CT findings will not be factored into clinical decision making. Rather, the clinical diagnostic CT will guide clinical decision making based on standard morphologic and contrast-enhancement criteria (LI-RADS®).

HCC Biopsy

US/CT-guided liver mass biopsy has been used in routine clinical practice for more than 20 years and has a well-documented safety profile. The major risk of HCC biopsy is bleeding, which occurs in less than 0.4% of HCC biopsies [18]. Another risk of HCC biopsy is needle track seeding. The risk of needle track seeding, once reported in up to 3% of HCC biopsies with older biopsy devices and techniques is now reported at 0% to 0.4% with modern biopsy devices and techniques [18]. Moreover, studies have shown that in patients with intermediate/advanced HCC who are not candidates for curative intent surgical resection or transplant, if needle tract seeding does occur it does not affect patient outcomes [18]. For the purposes of our study, only patients who meet our criteria of advanced HCC and without available archived tissue will be considered for clinical biopsy for other indications with PSMA stain performed from archived tissue stored in Tissue Registry. This may be done as a retrospective portion for correlation of existing archived tissue and PSMA IHC.

Research Peripheral Venipuncture/ Blood Draw
Venipuncture/blood draw is standard in clinical practice with a well-documented safety profile. The research blood draw will be performed in a clinical laboratory and paired with a clinical blood draw, when possible, to minimize the number of venipuncture events.

1.1.2 Potential Benefits

Evaluation of PSMA PET/CT in the patients with advanced HCC will potentially enable us to:
1. To establish ^{68}Ga-PSMA as an imaging biomarker that can predict response of HCC to novel immunotherapy,
2. Provide prognostic tools to personalize treatment decisions and thus, optimize the outcomes of these patients.

1.2 *Anticipated Duration of the Clinical Investigation*

The overall duration of this study in order to complete screening, enrollment, and all study procedures with follow-up for all patients will be approximately 3 years.

2 Study Objectives

In adult patients with advanced, pathologically confirmed HCC who are not amenable to curative restriction, transplantation or ablative therapies, and have radiographically measurable disease by RECIST; eligible for atezolizumab/bevacizumab front line therapy.

- **Specific Aim 1**. To test the performance of novel biomarkers derived from PSMA PET/CT to measure response compared to RECIST criteria, in advanced HCC patients treated with immunotherapy.

- **Specific Aim 2**. To identify precision imaging biomarkers that can predict response of HCC to novel immunotherapy.

- Correlative aim 2a: To assess biomarkers of response using extracellular vescicles and peripheral blood mononuclear cells.

3 Study Design

3.1 *General Design*

This is a prospective single-center study. A total of 30 patients who meet the inclusion and exclusion criteria will be enrolled to the study. All 30 patients will undergo ^{68}Ga-PSMA PET/CT imaging prior to initiation of immunotherapy to identify PSMA PET/CT positive patients (estimated N=20) for long term follow up. Patients will be recruited from the Mayo Clinic Cancer Center.

Patients may also be simutaneously enrolled in IRB 20-006433, EARLY DETECTION, ACCURATE STAGING, AND BIOLOGIC CHARACTERIZATION OF HCC WITH HYBRID 68GA-PSMA-DUAL-CONTRAST PET/MRI AND PET/CT USING CYCLOTRON-PRODUCED 68GA (PI: Dr. Ajit Goenka). In cases that patients are enrolled in both studies and are also undergoing a baseline 68Ga-PSMA PET/CT for IRB 20-006433; a repeat baseline 68Ga-PSMA PET/CT baseline scan does not have to be performed. Baseline imaging data for IRB 20-006433 will be used as the baseline timepoint for IRB 21-007799.

Patients who are PSMA PET/CT negative at baseline will continue their treatment per standard of care. Their data, from standard of care, will be collected as part of this study.

Patients who are PSMA PET/CT positive at baseline will receive up to a total of 5 scans during the course of study or until progression, whichever comes first. Each patient will undergo routine clinical blood workup including CBC, CMP, AFP, AFP-L3, INR and DCP as well as research blood collection at the time of scans (2 EDTAtubes, 2 NaHep tubes).

Patients will receive standard of care cross-sectional imaging (CT or MRI) and AFP testing every 3 cycles as per clinical protocol. Clinical variables will be collated and include age, sex, risk factors for HCC development, albumin, bilirubin, platelet count, Barcelona Clinic Liver Cancer stage, date of diagnosis, date of disease progression, any prior treatment history.

Test schedule for patients who are PSMA PET/CT positive at baseline:

Procedures	Prior to C1D1 of treatment	Treatment	End of Treatment	Event Monitoring every 6 months for 3 years
Electronic Medical Record Review	X	X	X	X
Eligibility Pre-Screening	X			
Informed Consent	X			
CBC, CMP, AFP, AFP-L3, DCP, INR	X	X	X	
Hep B and Hep C panel	X			
Blood (Bio-banking 2 EDTA, 2 NaHep tubes)	X	X	X	
Request external medical records when needed	X	X	X	X

Off Study Documentation*			X	
^{68}Ga-PSMA PET/CT	X*	X**		

*1 baseline treatment and scan
**End of every 3 cycles for a total of 4 scans during the 36 weeks

Blood Specimen Collection and Processing

Every effort will be made to collect peripheral blood at the time of routine blood draws for clinical visits/drug administration to limit patient morbidity and streamline patient care. If this is not possible, research blood collection will require a separate venipuncture at no charge to the patient.

Two (10ml) EDTA tubes, and 2 (10ml) NaHep tubes will be collected.

Blood samples will be sent to Rochester BAP lab. Blood must be sent ambient (NOT frozen). BAP will process the EDTA and Nahep tubes and stored frozen at -80C.

Biobanked blood will be used to assess for extracellular vesicles biomarkers (EVs) and peripheral blood mononuclear cells (PBMC). EVs are nano-objects (50-100 nm in diameter) in blood that hold promise as surveillance tools because they are numerous and stable.[19] Our team have developed a sensitive electrochemical immunoassay[20] well suited for detecting hepatic EVs carrying markers of steatosis, fibrosis and inflammation. We analyzed liver hepatitis-related biomarkers and identified a marker exhibiting more than 90% sensitivity and selectivity. Our study demonstrated higher sensitivity compared to conventional analytical methods for hepatic EV, such as ELISA or western blot, and showed significant increased expression of EpCAM and AFP on hepatic cancer EVs.[20, 21] We will apply this novel technology to detect pathology-informed markers in HCC EVs in circulation and evaluate the changes. We will assess the changes in peripheral blood cells, specifically NKG7$^+$ CD8$^+$ T cells and CX3CR1$^+$ Granzyme B$^+$ CD8$^+$ T cells as markers of response, particularly relevant to this study using ICI

Study Finances:

Tests and procedures that will be paid for by Benefactor funded grant including PET study (PET/CT), ^{68}Ga PSMA tracer production and IHC evaluation of soft tissue biopsy specimens with PSMA antibody. Other tests and procedures that are part of standard clinical care will be billed to the patients and/or to the applicable insurance.

Radiological analysis:

The PET scan will be reviewed by a Nuclear Radiologist. The maximum standard uptake value (SUVmax) at the lesion(s) will be obtained by manually placing a volume of interest (VOI) over the hepatic lesion, any other extrahepatic lesion that is deemed to be HCC-related and background liver. The PSMA accumulation at the site of the lesion will be scored based on visual interpretation of PSMA-activity relative to an internal reference standard based on the SUVmax threshold. The following data will be recorded: lesion(s) and background liver SUVmax and tumor to liver background ratio (TBR) SUVmax. An Abdominal Radiologist will review the cross-sectional imaging done as part of standard clinical care and document response both as a continuous variable (Δ tumor size, in cm), and in categories (CR-complete response; PR-partial response; SD-stable disease; PD-progressive disease) according to RECIST1.1. Response, per RECIST, will also be dichotomized as: 1) responders (CR and PR) versus non-responders (SD and PD); 2) disease control (CR, PR and SD) versus non-responders (PD). Metrics derived from ^{68}Ga-PSMA PET/CT will be compared with dichotomized RECIST categories. The Nuclear and Abdominal Radiologists will then review the images in consensus, blinded to the pathology findings.

Pathology:
Liver tissue samples will be processed per standard pathology protocols (formalin fixed, paraffin-embedded) and then stained with hematoxylin and eosin (H&E) and a previously validated monoclonal antibody targeting the extracellular domain of human PSMA in the Pathology Research Core (PRC). Tumor and background liver PSMA immunohistochemical grading will be performed by a hepatobiliary pathologist independent of the imaging findings as follows: 0 = no staining, trace = < 5% staining, 1= 5 to 30% staining, 2 = 31 to 60% staining and 3 = 61-100% staining. Additionally, HCC will be graded and staged as per standard Mayo Clinic protocols, including determination of tumor size, grade, vascular invasion, etc. as well as standard evaluation of background liver for fibrosis, inflammation, steatosis, etc. After independent grading, staining will be reviewed in consultation with a radiologist in order to match sites of histopathologic analysis to the lesion at cross-sectional imaging, but will be blinded to PET/CT findings.

3.2 *Primary Study Objectives*

A. Determine the time to response per RECIST
B. Determine PFS at 6 months per RECIST

4 Patient Selection, Enrollment and Withdrawal

4.1 *Inclusion Criteria*

- Patient with pathologically confirmed HCC not amenable to curative resection, transplantation or ablative therapies.
- Have radiographically measurable disease by RECIST.
- Eligible for atezolizumab/bevacizumab front line therapy.
- Male or female with age greater than 18 years, with the capacity and willingness to provide written informed consent.

4.2 *Exclusion Criteria*

- Pregnant and/or breast-feeding patients. A negative pregnancy test within 48 hours of the PET scan.
- Patients with higher than the weight/size limitations of PET/CT scanner.

4.3 *Patient Recruitment, Enrollment and Screening*

Patients will be recruited from Mayo Clinic Cancer Center. Scheduling calendar of these clinics and collaborating clinician referrals will identify patients meeting study requirements. Our study coordinator will contact potential candidates to discuss the study procedure, as well as its risks/benefits, and obtain informed written consent.

Patients will solely decide the waiting period between discussion and decision. The participant clinicians will address additional patient questions.

4.4 *Early Withdrawal of Patients*

4.4.1 When and How to Withdraw Patients

The patients may be withdrawn from the study prior to completion of all of the study related procedures if:

- Patient decides to withdraw from the study (withdrawal of consent).
- Patient suffers from a serious or life-threatening adverse effect due to the procedure.
- Patient fails to adhere to the protocol requirements.

4.4.2 Data Collection and Follow-up for Withdrawn Patients

Not applicable.

5 Study Procedures

The study will involve up to 5 visits for PSMA PET/CT, the initial imaging will be prior to systemic therapy and during the course of study (after 3, 6, 9, 12cycles of treatment), or until progression, whichever comes first. Long-term follow up after treatment includes 3 years or death, whichever comes first.

Investigational Drug

^{68}Ga-PSMA-HBED-CC is an investigational radiopharmaceutical that will be produced under cGMP in the Mayo Clinic Cyclotron Facility. At Mayo Clinic Rochester (MCR), we have received FDA approval (IND#151346) for research centered on the use of ^{68}Ga-PSMA in HCC.

5.1 Study Visits

PET/CT examination:
The patients enrolled into this study will undergo up to 5 PET/CT scan in Charlton 6-B. The PET/CT exam-related patient visit will last approximately 2.5 to 3.5 hours, including 30-40-minute nursing assessment, around 90-minutes for PSMA uptake, and 30 minutes for PET/CT scan. Scheduling for the exam will be completed by a study coordinator.

Participant preparation:
No fasting is required. Liberal fluids are encouraged.

Pre-Imaging Assessment:

Patients will undergo standard nursing assessment prior to the examinations as per Department of Radiology protocol.

During the ^{68}Ga-PSMA PET/CT exam:
In patients who are otherwise eligible for the study PET/CT will be performed as per standard PSMA PET/CT protocol at MCR. Patients will receive an injection of 5 ± 10% mCi of ^{68}Ga-PSMA. After the injection, patients will relax on a recliner in a quiet, dimly lit room for around 90 minutes while the PSMA circulates through the body. Scan coverage will extend from base of the skull to upper thighs. Bed position scan time will be dependent on the PET/CT scanner capabilities. At a minimum, 3 minutes per bed position will be used.

After completion of PET/CT examination:
The patient will be advised to drink plenty of water and stay near a rest room without extended travel for a few hours. The images will be stored on password protected Mayo Clinic computers.

Long Term Follow-up:

The patient will be contacted by telephone every 6 months for 3 year to ask about their health and medications.

6 Statistical Plan

Endpoint:
Aim 1: The endpoints are 1) time to treatment response per RECIST 1.1 and per PSMA PET/CT, defined as time from study regitration to CR/PR per RECIST 1.1 and per PSMA PET/CT; 2) time to progression per RECIST 1.1 and per PSMA PET/CT, defined as time from study registration to disease progression per RECIST 1.1 and per PSMA PET/CT.
Aim 2: The endpoint is PFS at 6-months post registration (PFS6) per RECIST 1.1.

Power Statement:
We powered this study to test the hypothesis that patients with positive PSMA PET/CT at baseline will have a shorter PFS, i.e. a lower rate of PFS6, compare to historical control where positive PSMA is defined as any lesion (within a patient) has a SUVmax > 4. The assumptions are that 2/3rd of the patients enrolled would have positive PSMA (i.e. N=20) and patients with negative PSMA would have PFS6 of 55% (historical data)[1]. Given 20 patients, we can detect a PFS6 difference of 26% (i.e. PFS6 drops from 55% to 29%) with 80% power assuming a one-sided p-value of 0.1 (using exact method).

Analysis Plan:
Aim 1: Time to reatment response per RECIST 1.1 and per PSMA PET/CT will be defined for each patients. The response per PSMA PET/CT will need to be 'confirmed' by CT finding. Specifically, if PSMA PET/CT identified a reduction of SUVmax which result in a response per PSMA PET/CT, the reduction needs to persist until there is a response per CT then the response per PSMA PET/CT is 'confirmed'. Time to response, per RECIST 1.1 and per PSMA PET/CT, will be correlated to each other using Cox proportional hazard model with response per

RECIST 1.1 as the dependent varible and the time to response per PSMA PET/CT will be included in the model as a time-vaying covariate.
Similar analysis plan applys to time to progression.

Aim 2: The PFS6 will be calculated among PSMA PET/CT positive patients using Kaplan-Meier [22] method with corresponding confidence interval calculated using Greenwood's formula. Since we will also gather data on PSMA negative patients, we will explore the potential prognostic effect of PSMA status on PFS using Cox proportional hazard model with PSMA PET/CT status (positive vs. negative) as the main variable of interest while adjusting for other potential confounders.

7 Safety and Adverse Events

7.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)
Any unanticipated problem or adverse event that meets the following three criteria:

- Serious: Serious problems or events that results in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others (including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, AND
- Unanticipated: (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, AND
- Related: A problem or event is "related" if it is possibly related to the research procedures.

Adverse Event
An untoward or undesirable experience associated with the use of a medical product (i.e. drug, device, biologic) in a patient or research subject.

Serious Adverse Event
Adverse events are classified as serious or non-serious. Serious problems/events can be well defined and include;

- death
- life threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- persistent or significant disability or incapacity
- substantial disruption of the ability to conduct normal life functions
- birth defect/congenital anomaly

and/or per protocol may be problems/events that in the opinion of the sponsor-investigator may have adversely affected the rights, safety, or welfare of the subjects or others, or substantially compromised the research data.

All adverse events that do not meet any of the criteria for serious, should be regarded as non-serious adverse events. All adverse events occurring during the study will be recorded in patient medical records. Records of these events will be maintained and reports submitted to IRB according to the regulatory requirements. Expected clinical adverse events and non-significant (not serious) clinical adverse events will not be reported. Expected clinical adverse events and anticipated adverse procedure effects are those listed in Section 1.1.1.

Adverse Event Reporting Period
For this study, the study treatment follow-up period is defined as 2 days after the PET/CT exam.

8 Data Handling and Record Keeping

8.1 *Confidentiality*

Information about study patients will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed patient authorization informing the patient of the following:

- What protected health information (PHI) will be collected from patients in this study?
- Who will have access to that information and why?
- Who will use or disclose that information?
- The rights of a research patient to revoke their authorization for use of their PHI.

In the event a patient revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of patient authorization. For patients that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the patient is alive) at the end of their scheduled study period.

8.2 *Source Documents*

The Source data comprise all information, original records of clinical findings, observations, or other activities in a clinical treatment necessary for the reconstruction and evaluation of the treatment response. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, radiological imaging data, memoranda, patients' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, patient files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the study.

8.3 *Records Retention*

The principal-investigator will maintain records and essential documents related to the conduct of the study. These will include patient case histories and regulatory documents.

The principal-investigator will retain the specified records and reports as outlined in the Mayo Clinic Research Policy Manual –"Retention of and Access to Research Data Policy" [redacted].

9 Study Finances

This study proposal has received funding approval from Benefactor funded grant.

10 Publication Plan

Findings will be shared and discussed with all of the investigators for the study. An estimated timeline for the creation of an abstract will be defined at that time. An abstract of the completed study, after input from all of the authors, will be submitted to a radiology, hepatology and/or hepatobiliary surgery meeting. A manuscript of the study, having received input from all of the authors will be submitted to a peer-reviewed journal.

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