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Hyperpolarized C-13 Pyruvate as a Biomarker of PI3K/mTOR Pathway Inhibition in Patients with Advanced Solid Tumor Malignancies

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Protocol Signature Page

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1. I agree to follow this protocol version as approved by the UCSF Protocol Review Committee (PRC), Institutional Review Board (IRB), and Data Safety Monitoring Committee (DSMC).
2. I will conduct the study in accordance with applicable IRB requirements, Federal regulations, and state and local laws to maintain the protection of the rights and welfare of study participants.
3. I certify that I, and the study staff, have received the requisite training to conduct this research protocol.
4. I agree to maintain adequate and accurate records in accordance with IRB policies, Federal, state and local laws and regulations.

UCSF Principal Investigator / Study Chair

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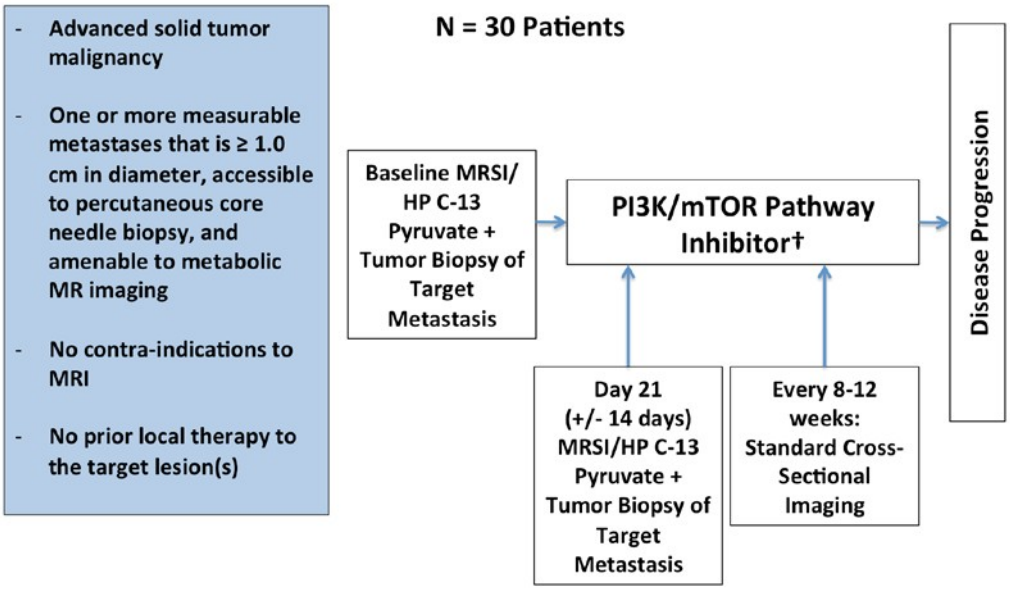
Printed Name

_____ _____
Signature Date

Abstract

Title	**Hyperpolarized C-13 Pyruvate as a Biomarker of PI3K/mTOR Pathway Inhibition in Patients with Advanced Solid Tumor Malignancies**
Patient population	Subjects will be enrolled in two sequential phases: • Part A: Feasibility Run-In (N =50 patients). Patients with advanced solid tumor malignancies with at least one liver or other intra-abdominal metastasis will be enrolled with iterative adjustment of coil design to optimize imaging parameters including spatial resolution and signal-to-noise ratio (SNR) of hyperpolarized pyruvate/lactate within the target metastatic lesion(s). • Part B: Biomarker Cohort (N = 30 patients). Patients with advanced solid tumor malignancies and the presence of at least one liver or other intra-abdominal metastasis amenable to hyperpolarized C-13 pyruvate metabolic MR imaging who are planning on being treated with agent targeting PI3K/mTOR pathway will be enrolled. Exclusion criteria: • Patients unwilling or unable to undergo MR imaging, including patients with contraindications to MRI, such as cardiac pacemakers or non-compatible intracranial vascular clips.
Background and Rationale for Study	Dynamic nuclear polarization significantly enhances the signal-to-noise ratio of magnetic resonance (MR) spectroscopic imaging by 10,000-fold, enabling the visualizing of metabolic activity in cancer cells using hyperpolarized probes. PI3K/mTOR-mediated expression of lactate dehydrogenase catalyzes a key reaction as part of the glycolytic process, namely the conversion from pyruvate to lactate. Thus, by injecting hyperpolarized ^{13}C pyruvate, one can quantify the intra-tumoral conversion rate of pyruvate to lactate using metabolic MR imaging in real-time, thereby potentially serving as a surrogate functional biomarker of PI3K/mTOR pathway activity. This hypothesis has been supported by pre-clinical models in which pyruvate-to-lactate conversion visualized on metabolic MR imaging was correlated with tissue–based assays of LDH enzymatic activity, and down-regulation of PI3K/mTOR activity (e.g. everolimus) led to significant down-regulation of pyruvate-to-lactate conversion, an effect that was not observed with non-specific cytotoxic agents (eg. temozolomide). These data support the utility of hyperpolarized ^{13}C pyruvate/metabolic MR imaging as a functional biomarker of PI3K/mTOR pathway activity. A phase 1 study of hyperpolarized ^{13}C pyruvate in patients with localized prostate cancer has demonstrated safety and feasibility of this approach. Since completion of this phase 1 study, there have been significant technological enhancements to enable broader applicability of hyperpolarized ^{13}C metabolic MR imaging, including use of an automated polarizer and construction of novel radiofrequency coils to enable imaging of other anatomic sites besides the prostate, including the liver and other intra-abdominal organs. We have recently completed a pilot study designed to optimize radiofrequency coil geometry enabling visualization of liver anatomy with high spatial resolution. The current study is designed to clinically investigate the utility of hyperpolarized ^{13}C pyruvate/metabolic MR imaging as a functional imaging biomarker of PI3K/mTOR pathway activity. In the feasibility run-in phase (Part A; N =50 pts), patients with advanced solid tumor malignancies of any tumor type with liver or other intra-abdominal metastases will be enrolled, with iterative adjustment of coil geometry and imaging sequences to optimize the signal-to-noise ratios of intra-tumoral hyperpolarized pyruvate and lactate signal within the liver or other intra-abdominal

	metastase(s). Subsequently, during Part B of the study (N = 30 pts), patients with advanced solid tumor malignancies who possess at least one liver or intra-abdominal metastatic lesion amenable to metabolic MR imaging, who are also planning on undergoing systemic treatment with a PI3K/mTOR pathway inhibitor, will be enrolled. Patients will undergo baseline imaging and repeated after approximately 21 days of systemic therapy with PI3K/mTOR pathway inhibitor, to assess for change from baseline in intra-tumoral pyruvate-to-lactate metabolic flux. Patients will undergo optional paired core needle tumor biopsies to correlate imaging findings with tissue-based markers of pyruvate metabolism and the PI3K/mTOR pathway. Promising results from the current study supporting the utility of hyperpolarized C-13 pyruvate imaging as a potential biomarker of target inhibition would support its future integration in early phase clinical studies as a companion imaging biomarker to optimize dose selection and as a surrogate measure of on-target tumor response.
Primary Objectives	Part A: To optimize the signal-to-noise ratio in detecting intra-tumoral hyperpolarized C-13 lactate/pyruvate signal within liver or other intra-abdominal metastases using metabolic MR imaging in patients with advanced solid tumor malignancies. Part B: To determine the mean percent change from baseline in peak intra-tumoral hyperpolarized lactate to pyruvate ratio after initiation of treatment with PI3K/mTOR pathway inhibitor.
Secondary Objectives	• To investigate for an association between percent change from baseline in peak intra-tumoral hyperpolarized lactate to pyruvate ratio after initiation of treatment with PI3K/mTOR pathway inhibitor with subsequent clinical outcomes including objective response rate, clinical benefit rate, and progression-free survival. (Part B) • To characterize further the safety profile of hyperpolarized C-13 pyruvate injections • To determine the reproducibility of intra-tumoral HP lac/pyr ratio with same-day repeated dose studies.
Exploratory Objective (Part B)	• To investigate for an association between intra-tumoral hyperpolarized lactate to pyruvate ratio with histologic markers of PI3K/mTOR pathway and glycolytic metabolism including phosphorylated S6, pAKT, and LDH expression. (Part B; optional)

Study Design	This is a single center prospective imaging study investigating the utility of hyperpolarized C-13 pyruvate/metabolic MR imaging. The current protocol will serve as a companion imaging biomarker study paired with therapeutic trials of PI3K/mTOR pathway inhibitors (e.g. CUDC-907, BYL719), as well as a stand-alone protocol for patients treated with standard-of-care therapies inhibiting the PI3K/mTOR signaling pathway (eg. everolimus). In Part A (run-in feasibility phase), patients will undergo imaging at a single time point, without paired tumor biopsy. There will be no follow up imaging or requirement for treatment with PI3K/mTOR pathway inhibitor. Iterative adjustment of radiofrequency coil geometry and imaging sequences will be undertaken to optimize intra-tumoral hyperpolarized pyruvate/lactate signal-to-noise ratio. In part B, patients will undergo paired baseline hyperpolarized C-13 pyruvate imaging + tumor biopsy, then initiate treatment with agent inhibiting the PI3K/mTOR pathway. After 21 days (+/- 14 days), patients will undergo repeat hyperpolarized C-13 pyruvate MR imaging + tumor biopsy. Patients will subsequently be treated with PI3K/mTOR pathway inhibitor until disease progression, unacceptable toxicity, or patient/physician decision to discontinue therapy. **Part B Study Schema** 
Number of patients	50 patients will be accrued during the feasibility run-in period (Part A). 30 patients will be accrued during the main biomarker investigational period (Part B).
Duration of Therapy	In Part A, patients will undergo imaging at a single time point without paired tumor biopsy. In Part B, patients will undergo paired hyperpolarized pyruvate/metabolic MR imaging + tumor biopsy at baseline and after approximately 21 days of therapy.

Duration of Follow up	In Part A, no follow up is planned. In Part B, patients will be followed until discontinuation of treatment with PI3K/mTOR pathway inhibitor.
Duration of study	The anticipated accrual period is 24 months (~ 1.5 patients/month). Duration of follow up will vary by tumor type but is expected to average approximately 6 months, leading to an estimated total study duration of 30 months.
Imaging Agents	Hyperpolarized ^{13}C pyruvate

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1 Background

1.1 Study Rationale

Dynamic nuclear polarization significantly enhances by 10,000-fold the signal-to-noise ratio of magnetic resonance (MR) spectroscopic imaging, enabling the visualizing of metabolic activity in cancer tissue using hyperpolarized metabolic probes including pyruvate **(Figure 1).**[1] Phosphatidylinositol 3-kinase (PI3K)/mammalian target of Rapamycin (mTOR)-mediated expression of lactate dehydrogenase (LDH) catalyzes the conversion from pyruvate to lactate. This is a key step in aerobic glycolysis, a hallmark of cancer cells. By injecting hyperpolarized ^{13}C pyruvate, one can quantify in real-time using metabolic MR imaging the intra-tumoral conversion rate of pyruvate to lactate, thereby potentially serving as a surrogate functional biomarker of PI3K/mTOR pathway activity **(Figure 2).** This hypothesis has been supported by pre-clinical models in which pyruvate-to-lactate conversion visualized on metabolic MR imaging was correlated with tissue –based assays of LDH enzymatic activity, and down-regulation of PI3K/mTOR activity (eg. everolimus) led to significant down-regulation of pyruvate-to-lactate conversion, an effect that was not observed with non-specific cytotoxic agents (eg. temozolomide). These data support the utility of hyperpolarized C-13 pyruvate/metabolic MR imaging as a functional biomarker of PI3K/mTOR pathway activity.

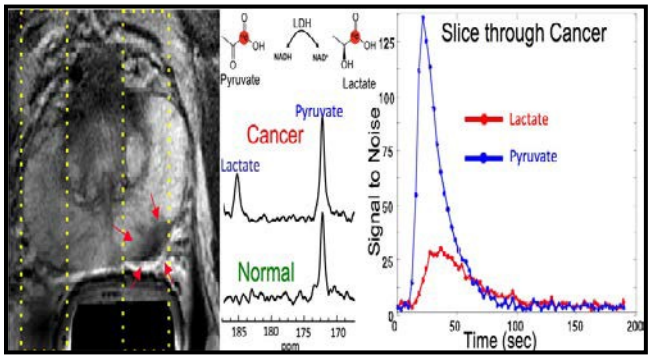


Figure 1. ^{13}C-pyruvate uptake within 15-20s with high conversion in regions including biopsy-proven cancer. These studies demonstrated high signal-to-noise ratio compared with standard proton spectroscopy.

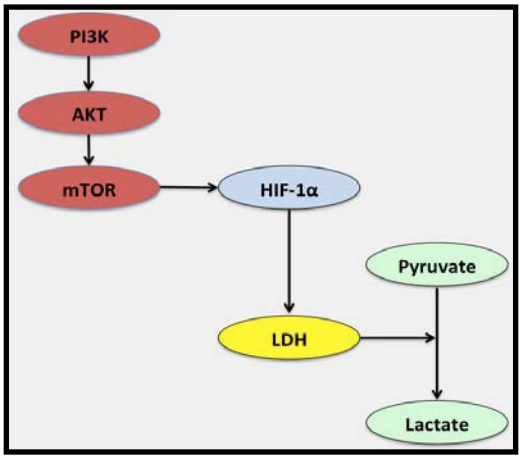


Figure 2. Link between PI3K/mTOR pathway and Pyruvate Metabolism via HIF-1 alpha and LDH.

A phase 1 study of hyperpolarized ^{13}C pyruvate in patients with localized prostate cancer has demonstrated safety and feasibility of this approach.[2] Since completion of this phase 1 study, there have been significant technological enhancements to enable broader applicability of hyperpolarized ^{13}C metabolic MR imaging, including use of an automated polarizer and construction of novel radiofrequency coils to enable imaging of other anatomic sites besides the prostate, including the liver and other intra-abdominal organs.

The current study is designed to clinically investigate the utility of hyperpolarized C-13 pyruvate/metabolic MR imaging as a functional imaging biomarker of PI3K/mTOR pathway activity. In the feasibility run-in phase (Part A; N = 50 pts), patients with advanced solid tumor malignancies of any tumor type will be enrolled, with iterative adjustment of coil geometry and imaging sequences to optimize the signal-to-noise ratio of intra-tumoral hyperpolarized pyruvate and lactate signal. Subsequently, during Part B of the study (N = 30 pts), patients with advanced solid tumor malignancies who possess at least lesion amenable to metabolic MR imaging, who are also planning on undergoing systemic treatment with a PI3K/mTOR pathway inhibitor, will

be enrolled. Patients will undergo baseline imaging and repeated after approximately 21 days of systemic therapy with PI3K/mTOR pathway inhibitor, to assess for change from baseline in intra-tumoral pyruvate-to-lactate metabolic flux. Patients will undergo optional paired tumor biopsies to correlate imaging findings with tissue-based markers of pyruvate metabolism and the PI3K/mTOR pathway.

Promising results from the current study supporting the utility of hyperpolarized C-13 pyruvate imaging as a potential biomarker of target inhibition would support its future integration in early phase clinical studies as a companion imaging biomarker to optimize dose selection and as a surrogate measure of on-target tumor response.

1.2 C-13 pyruvate serves as biomarker of PI3K/mTOR pathway in pre-clinical models

Pre-clinical studies of breast and brain tumor xenografts treated with the established mTOR (TORC1) inhibitor everolimus demonstrated significant down-regulation of hyperpolarized pyruvate-to-lactate conversion detected by HP ^{13}C MRI as early as two days after treatment initiation (**Figure 3**).[3,4] In the preclinical model, declines in HP lactate/pyruvate signal within the tumor were observed prior to changes in tumor size, were associated with subsequent tumor growth stabilization, correlated with intra-tumoral immunohistochemical evidence of reductions in PI3K/mTOR pathway activity including decreased LDH and HIF-1α expression, and were not seen with treatment with a non-specific DNA damaging agent (temozolomide). These pre-clinical observations demonstrate that HP ^{13}C MRI can provide early and specific evidence of effective PI3K/mTOR pathway inhibition.

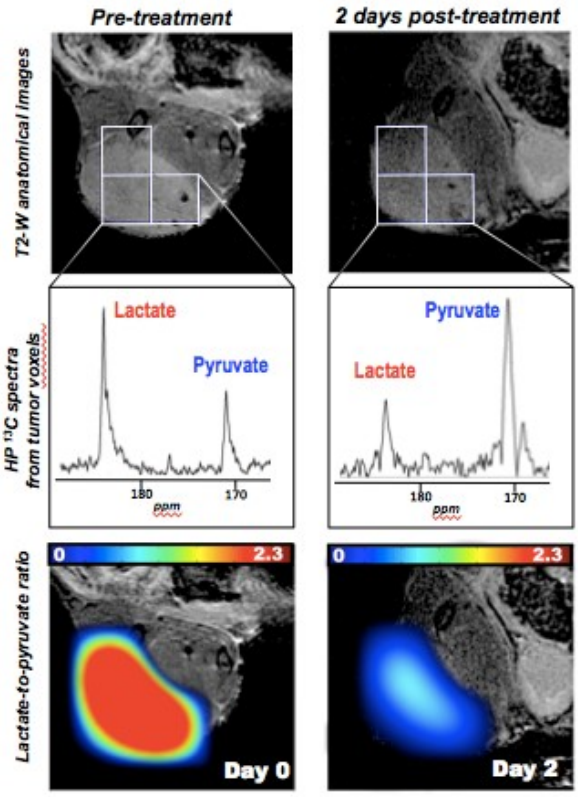


Figure 3. PI3K/mTOR signal inhibition with everolimus leads to early declines in HP ^{13}C pyruvate to lactate conversion in subcutaneous glioblastoma tumors (GS2 cell line with PTEN loss) prior to changes in tumor size.

1.3 Previous Clinical Experience with Hyperpolarized C-13 Pyruvate

As a first step in evaluating the use of pyruvate injection in humans, a phase 1, placebo-controlled study was conducted to evaluate the safety of ascending doses in healthy volunteers between 18 and 45 years of age. As no imaging was performed in this study, pyruvate injection produced using [1-^{13}C]pyruvate was used. This study was completed in December 2007. In the original protocol it was planned that doses up to 0.71 ml/kg body weight would be given,

providing there was no evidence of significant investigational medicinal product (IMP)-related adverse events (AEs) at lower dose levels.

In each cohort of 6 subjects, 4 subjects received pyruvate injection and 2 received saline. Doses up to 0.43 ml/kg body weight were well tolerated and no IMP-related AEs were observed that warranted unblinding the study to reveal which subjects had received Pyruvate Injection versus placebo. However, in the 0.57 ml/kg body weight cohort, 2 of 6 subjects showed IMP-related non-serious AEs (“unresponsiveness" in subject 001-0026 and “flushing" accompanied by changes in blood pressure and heart rate in subject 001-0030) that the principal investigator considered to be concerning and related to the administration of IMP. The study was unblinded for this cohort and it was ascertained that the events of concern occurred in subjects who had received pyruvate injection.

The event described as “unresponsiveness” was NOT unresponsiveness as it is generally defined. Instead, the patient remained conscious with stable vital signs but did not answer a question immediately when asked (it took seconds longer than expected). The “flushing” event, accompanied by changes in systolic and diastolic blood pressure and heart rate, was thought to be indicative of a “baroreflex/hemodynamic” response. All events in both subjects resolved spontaneously without any treatment or intervention. The events were reviewed by medically qualified sponsor representatives and it was decided not to proceed with the next planned dose level of 0.71 ml/kg. The protocol was amended to repeat the 0.43 ml/kg dose, to confirm that it was as well tolerated in a second cohort of 6 healthy volunteers as it was in the first cohort. The introduction of additional safety monitoring was considered, but none was found to be relevant or necessary as comprehensive and intense safety monitoring was already being applied. Data from the second 0.43 ml/kg dose group confirmed that this dose was well tolerated.

No serious adverse events (SAEs) occurred in the study and all other non-serious AEs that occurred in any subject throughout the study were mild in intensity, short-lasting, and resolved spontaneously without any treatment or intervention. Throughout the study, data on serum biochemistry variables and post-dosing changes were unremarkable, and no notable changes in vital signs, hematology, urinalysis, electrocardiogram (EKG) variables or other safety variables were registered.

A similar, phase 1, placebo-controlled study with doses up to 0.43 ml/kg Pyruvate Injection was conducted in elderly (60 years of age) volunteers. All doses up to 0.43 ml/kg body weight were very well tolerated and no significant or serious AEs were reported. All the non-serious AEs that occurred were mild in intensity, short-lasting and resolved spontaneously without treatment or intervention. No true “unresponsiveness", unresponsiveness as defined in the prior study, flushing, or other events of concern occurred. Throughout the study, data on serum biochemistry variables and post-dosing changes were unremarkable, and no notable changes in vital signs, hematology, EKG variables or other safety variables were registered.

On the basis that doses of pyruvate injection up to 0.43 ml/kg body weight were shown to be safe and well tolerated in young healthy volunteers and elderly volunteers, a phase I dose escalation study was undertaken at UCSF in men with localized prostate cancer who were on active surveillance or were pre-local therapy. In this study, three dose levels of pyruvate injection, 0.14 ml/kg body weight, 0.28 mg/kg body weight, and 0.43 ml/kg body weight were evaluated. Thirty-one patients underwent successful injection and imaging.

Dose limiting toxicity was defined as any grade 2 or higher toxicity (excluding asymptomatic lab abnormalities). Dose limiting toxicity is usually defined as grade 3 toxicity. However, given this is an imaging agent rather than a therapeutic agent, the threshold was set lower in this study.

There were no dose-limiting toxicities or toxicities deemed to be clinically significant. All possibly, probably, or definitely related toxicities are listed in the table below:

Table 1: Possibly, probably, or definitely related toxicities: Phase I Study at UCSF

Dose level	n	Toxicity	Grade
1 (0.14 mL/kg)	6	Orange urine	1
		Pharmaceutical smell	1
		Pruritus	1
2 (0.28 mL/kg)	6	Cold sensation with injection	1
		Dysgeusia	1
3 (0.43 mL/kg)	19	Dizziness	1
		Dysgeusia (4)	1
		Fatigue	1
		Hypocalcemia	1
		Hypokalemia	1
		Hypotension	1
		Nausea	1
		Pain - headache	1
		Smell change (2)	1
		Sore throat	1
		Diarrhea	2

The one episode of grade 2 diarrhea was thought to be more likely related to the enema required prior to the endorectal coil placement for the MR, but its relationship to the pyruvate injection could not be ruled out. The hypotension and dizziness occurred one day after the pyruvate injection in a patient who took a dose of metoprolol without checking his blood pressure prior (when he generally checks it prior to taking antihypertensives). Therefore, it was thought unlikely to be related, but a relationship to pyruvate injection could not definitively be ruled out.

The maximum administered dosage 0.43 mL/kg was established as the phase 2 dosage. Higher doses were not evaluated for three reasons: 1) the volume required to administer a higher dose would lengthen the time from the start of injection until when the imaging could be initiated, affecting the polarization, 2) the imaging at a dosage of 0.43 mL/kg was of sufficient

quality to indicate that a higher dosage is not needed, 3) the aforementioned study raised concern for toxicity at higher doses (although this is questionable based on the original data).

The concerns for toxicity based on the earlier phase I experience prompted intensive monitoring during the UCSF phase I study, including continuous lead II EKG during and for ten minutes following the injection, serial EKGs for two hours following the injection, laboratory and clinical monitoring for two hours following the injection, and follow-up both one and seven days following the injection. This monitoring did not yield any safety concerns.

1.4 Expanding the anatomic coverage of metabolic MR imaging

To enable broader applicability of hyperpolarized ^{13}C metabolic MR imaging, we have recently completed a pilot study in healthy volunteers (CC# 149516) evaluating the use of newly constructed radiofrequency coils to enable imaging of the liver and other intra-abdominal organs. A representative image of the liver captured by the new radiofrequency coil is shown in **Figure 4.** A summary of the observed signal-to-noise ratio achieved with the various coil geometries using two MR pulse sequences (SSFSE and LAVA) is shown in **Figure 5.** The medium size flex coil gave the best SNR for up to 7cm deep in the liver and will be the starting coil geometry for the planned study.

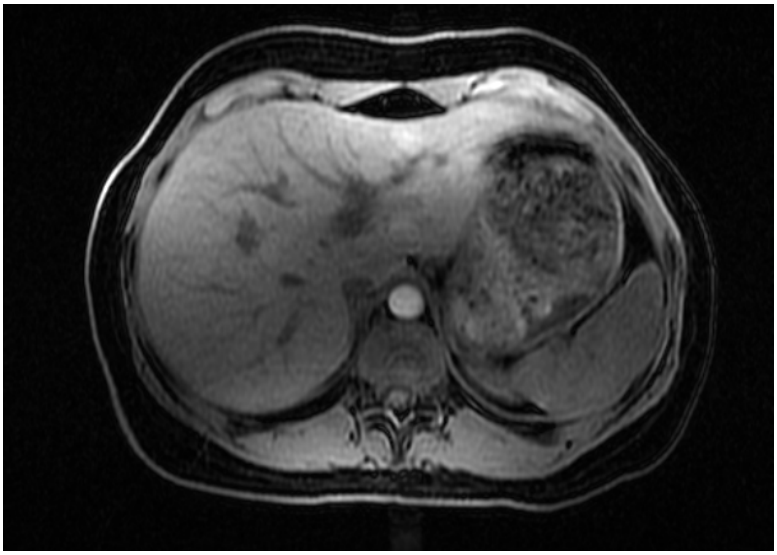


Figure 4. A representative image of the liver in a healthy volunteer using the newly fabricated radiofrequency coils with capability for metabolic MR imaging with HP probes including C-13 pyruvate.

SSFSE	SNR 1	SNR 2	SNR 3	SNR 4
Small Flex	41	27	30	13
Medium Flex	40	26	29	13
Large Flex	33	26	30	15
Body Coil	29	33	28	30

LAVA	SNR 1	SNR 2	SNR 3	SNR 4
Small Flex	236	140	162	64
Medium Flex	355	214	245	102
Large Flex	310	198	258	111
Body Coil	149	162	155	158

Figure 5. Average signal-to-noise ratios in the liver using the various sizes of radiofrequency coils. The medium flex coil resulted in the best signal-to-noise ratio up to 7 cm deep from the skin surface in the liver.

Rationale for Use of Multiple injections of Hyperpolarized [1-^{13}C]pyruvate and/or [2-^{13}C] pyruvate

When [2-^{13}C]pyruvate, is used instead of [1-^{13}C]pyruvate, the ^{13}C-label is incorporated into TCA (tricarboxylic acid) cycle intermediates rather than being released as $^{13}CO_2$ (Figure 1). Combined with the fact that GMP [2-^{13}C]pyruvate has been synthesized, has the same safety profile as [1-^{13}C]pyruvate, and has been shown to be useful in monitoring TCA metabolism in pre-clinical studies of the heart (1), brain (2) and prostate makes [2-^{13}C]pyruvate an attractive substrate for clinical translation. In the perfused rat heart (1), the conversion of HP pyruvate to [2-^{13}C]lactate, [1-^{13}C]acetyl-carnitine, [1-^{13}C]citrate and [5-^{13}C]glutamate was observed, and following cardiac ischemia, the TCA cycle intermediates, [1-^{13}C]citrate and [5-^{13}C]glutamate, decreased and [2-^{13}C]lactate increased (1). In the rat brain, increasing the HP pyruvate injection concentration to 125mM led to the observation of multiple TCA cycle intermediate metabolites including [5-^{13}C]glutamate and [1-^{13}C]citrate (2) indicating that pyruvate was getting across the blood brain barrier in sufficient time for metabolism to occur. By increasing PDH activity through DCA (dichloroacetate) stimulation, detection of HP [1-^{13}C]acetyl carnitine signal, along with a 40% increase in HP [5-^{13}C]glutamate were observed (2). For studying prostate cancer, we have shown in *ex vivo* patient derived prostate cancer tissue slice studies that ^{13}C labeling of both the C-1 and C-2 positions of pyruvate provides a way to assess both up-regulated Warburg effect (HP ^{13}C lactate) and TCA cycle (HP ^{13}C glutamate) in prostate cancer. However, the utility of [1,2-^{13}C]pyruvate is limited by lower SNR due to ^{13}C-^{13}C coupling and overlap of the coupled resonances. Therefore, initial clinical translation will involve the use of HP ^{13}C MRI protocols and employ sequential injections of [1-^{13}C]pyruvate and/or [2-^{13}C]pyruvate, thereby alleviating the problems associated with doubly labeled [1,2-^{13}C]pyruvate. In addition to improving the metabolic information provided by injecting [1-^{13}C]pyruvate alone, the second pyruvate injection, using either [1-^{13}C]pyruvate or [2-^{13}C]pyruvate, could also provide an expanded region of coverage (i.e. the ability to assess cancer lesions that were outside of the FOV of the first HP C-13 pyruvate MRI scan), and/or reproducibility of pyruvate metabolism (key to understanding the significance of metabolic changes with cancer aggressiveness and response to therapy). To accomplish the acquisition of this important metabolic data, patients will have the option of undergoing repeated dose imaging studies of HP C-13 pyruvate, up to two injections per time point, separated by 15-60 minutes.

1.5 Safety of Repeated HP C-13 Pyruvate Injections

There is ample evidence from pre-clinical studies and from the known short half-life of pyruvate metabolism to support the safety and feasibility of this approach. Specifically, hundreds of IUCAC approved pre-clinical ^{13}C MRI studies (mice, rats and canines) at UCSF have used multiple injections of hyperpolarized [1-^{13}C]pyruvate within the same imaging study without evidence of adverse events[5,6]. The SpinLab DNP polarizer, used in patient studies, allows for up to 4 samples to be polarized and dissolved in rapid succession. We have shown in a rat model that minimal perturbations occurred in pyruvate metabolism as a result of 4 injections of hyperpolarized [1-^{13}C]pyruvate injected in 5 minute intervals. Moreover, two injections of hyperpolarized [1-^{13}C]pyruvate have already occurred in patient studies. These studies have demonstrated the safety of serial injections without any adverse effects noted.

2 Study Objectives

2.1 Primary Objectives

Part A:

To optimize the signal-to-noise ratio in detecting intra-tumoral hyperpolarized C-13 lactate/pyruvate signal within liver or other intra-abdominal metastases using metabolic MR imaging in patients with advanced solid tumor malignancies.

Part B:

To determine the mean percent change from baseline in peak intra-tumoral hyperpolarized lactate to pyruvate ratio after initiation of treatment with PI3K/mTOR pathway inhibitor.

2.2 Secondary Objectives

- To investigate for an association between percent change from baseline in peak intra-tumoral hyperpolarized lactate to pyruvate ratio after initiation of treatment with PI3K/mTOR pathway inhibitor with subsequent clinical outcomes including objective response rate, clinical benefit rate, and progression-free survival. (Part B)
- To characterize further the safety profile of hyperpolarized C-13 pyruvate injection
- To determine the reproducibility of intra-tumoral HP lac/pyr ratio with same-day repeated dose studies.

2.3 Exploratory Objective (Part B)

To investigate for an association between intra-tumoral hyperpolarized lactate to pyruvate ratio with histologic markers of PI3K/mTOR pathway and glycolytic metabolism including phosphorylated S6, pAKT, and LDH expression. (Part B; optional)

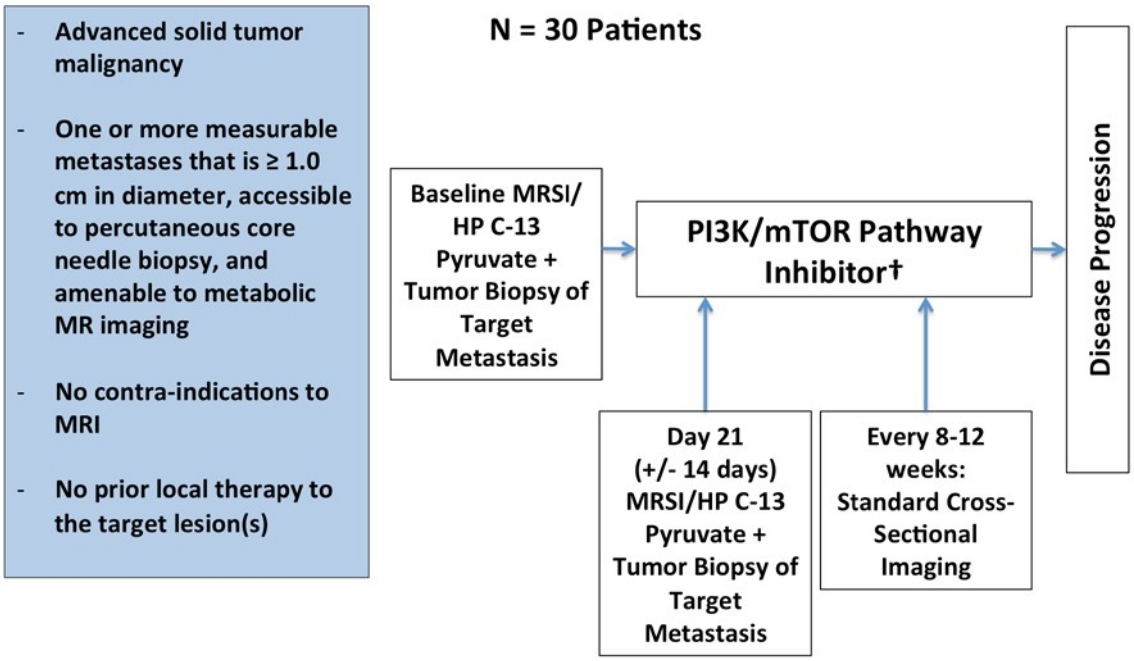
3 Study Design

3.1 Characteristics

This is a single center prospective imaging study investigating the utility of hyperpolarized C-13 pyruvate/metabolic MR imaging. The current protocol will serve as a companion imaging biomarker study paired with therapeutic trials of PI3K/mTOR pathway inhibitors (e.g. CUDC-907, BYL719) as well as a stand-alone protocol for patients treated with standard-of-care PI3K pathway targeting therapies (eg. everolimus).

In Part A (run-in feasibility phase), patients will undergo imaging at single time point, without paired tumor biopsy. There will be follow up imaging or requirement for treatment with PI3K/mTOR pathway inhibitor. Iterative adjustment of radiofrequency coil geometry and imaging sequences will be undertaken to optimize intra-tumoral hyperpolarized pyruvate/lactate signal-to-noise ratio during Part A of the study.

In Part B, patients will undergo paired baseline hyperpolarized C-13 pyruvate imaging + optional tumor biopsy, then initiate treatment with agent inhibiting the PI3K/mTOR pathway. After 21 days (+/- 14 days), patients will undergo repeat hyperpolarized C-13 pyruvate MR imaging + optional paired tumor biopsy. Patients will subsequently be treated with PI3K/mTOR pathway inhibitor until disease progression, unacceptable toxicity, or patient/physician decision to discontinue therapy.



3.2 Number of Subjects

Part A: 50 patients with advanced solid tumor malignancies with at least one metastatic liver or intra-abdominal lesion amenable to hyperpolarized C-13 pyruvate/metabolic MR imaging will be enrolled.

Part B: 30 patients with advanced solid tumor malignancies with at least one at least metastatic lesion amenable to percutaneous biopsy and metabolic MR imaging who are planning on being treated with PI3K/mTOR pathway inhibitor will be enrolled.

3.3 Eligibility Criteria

Inclusion Criteria:

- Presence of at least one target liver or other intra-abdominal lesion detected by standard staging scans that, in the judgment of Study Investigators, would be amenable to hyperpolarized C-13 pyruvate/metabolic MR imaging:
    - Target lesion must measure ≥1.0 cm in long axis diameter on CT or MRI
- The subject is able and willing to comply with study procedures and provide signed and dated informed consent.
- Eastern Cooperative Oncology Group (ECOG) Performance Status of 0 or 1 (see Appendix 1).
- Adequate organ function, including creatinine < 1.5 x ULN or estimated creatinine clearance ≥50 mL/min (by the Cockcroft Gault equation) and total bilirubin <3x ULN.

Part B only:

- No prior local therapy to target lesion.
- If patient agrees to optional biopsy:
    - Presence of at least one target lesion amenable to percutaneous tumor biopsy in the judgment of Interventional Radiology
    - No history of bleeding diathesis.
    - Patients on anti-coagulation they must be able to safely stop treatment for purposes of tumor biopsy.
- Planned treatment with agent targeting PI3K/mTOR pathway (either standard of care or investigational agent)

Exclusion Criteria

- Patients who because of age, general medical or psychiatric condition, or physiologic status cannot give valid informed consent.
- Patients unwilling or unable to undergo MR imaging, including patients with contra-indications to MRI, such as cardiac pacemakers or non-compatible intracranial vascular clips.
- Metallic implant or device that distorts local magnetic field and compromises the quality of MR imaging.
- Poorly controlled hypertension, defined as systolic blood pressure at study entry greater than 160 mm Hg or diastolic blood pressure greater than 100 mm Hg. The addition of anti-hypertensives to control blood pressure is allowed.
- Congestive heart failure or New York Heart Association (NYHA) status ≥ 2.
- A history of clinically significant EKG abnormalities, including QT prolongation (QTcF > 500 ms), a family history of prolonged QT interval syndrome, or myocardial infarction (MI) within 6 months of study entry. Patients with rate-controlled atrial fibrillation/flutter will be allowed on study.
- Any condition that, in the opinion of the Principal Investigator, would impair the patient's ability to comply with study procedures.

3.4 Study Timeline

In Part A, no follow up is planned.

In Part B, patients will be followed until discontinuation of treatment with PI3K/mTOR pathway inhibitor.

The anticipated accrual period is 24 months (~ 1-2 patients/month). Duration of follow up will vary by tumor type but is expected to average approximately 6 months, leading to an estimated total study duration of 30 months.

3.5 Study Termination

The principal investigator reserves the right to terminate the study at any time.

Termination of the study will be considered in the event of any safety concerns arising at any time during the performance of the study.

If it becomes necessary to consider termination of the study after dosing has begun, dosing may be suspended pending discussion between the investigators and the DSMC.

4 Investigational Medicinal Product

4.1 Description, Supply and Storage of Investigational Medicinal Product

4.1.1 Hyperpolarized C-13 Pyruvate

^{13}C is a stable, non-radioactive isotope of carbon with approximately 1% natural abundance.C-13 pyruvate has exactly the same chemical characteristics as pyruvate. In C-13 pyruvate, either the C-1 or C-2 atom has been replaced by a ^{13}C-nucleus, which has a magnetic moment and can be hyperpolarized in the presence of an EPA, i.e., AH111501 sodium salt (a stable trityl radical) by DNP. As C-13 pyruvate has the same chemical characteristics as pyruvate, it is metabolized the same way. However, the fact that it can be hyperpolarized means that MRSI can be used to rapidly detect the hyperpolarized ^{13}C-label in C-13 pyruvate and its metabolites, alanine, lactate, bicarbonate, and other metabolites of the TCA cycle such as glutamate.

Formulation

The formulation of IMP in this study will be compounded using 250 mM C-13 pyruvate and up to 3 μM AH111501 sodium salt. The osmolality of this formulation is ~500 mosmol/kg. After compounding is completed, a sterile fluid path will be pre-filled with the IMP under aseptic conditions. The sterile fluid path itself will be manufactured and cleaned under aseptic conditions in accordance with GMP guidelines (ISO level 7). The pre-filled sterile fluid path and automated hyperpolarizer/quality control instrument (GE SPINLab^{TM}) placed in an adjacent room to the MR suite, will then be used to generate the final hyperpolarized C-13 pyruvate contained within a syringe for rapid injection into the patient (see preparation methods below).

The IMP will be intravenously injected at a rate of 5 ml/second followed by a 20-ml saline flush at 5 ml/second. Prior to dosing, the injection line will be primed with saline solution and some of this will be flushed into the subject ahead of the IMP. In the case of unforeseen events, such as difficulties with the power injector, etc., hand injection will be allowed.

Supply and Packaging

Shipments containing kit supplies for IMP compounding will be provided by the manufacturer and shipped from their clinical supply unit. Site personnel at the clean room will be responsible for receiving kit supplies, compounding the IMP, filling the sterile fluid path using aseptic technique, performing quality control using the GE SPINLab^{TM} instrument, and performing drug accountability. See preparation methods (below) for more information.

Storage

The investigators are responsible for ensuring that deliveries of IMP and other study materials from the manufacturer are correctly received, recorded, handled, and stored safely and properly in accordance with all applicable regulatory guidelines, and used only in accordance with this protocol. The pre-filled sterile fluid paths will be stored at $\leq$ -20 degrees C, and thawed prior to clinical use.

Preparation Methods

IMP will be compounded under the supervision of a trained pharmacist, using a mixture of ^{13}C-pyruvic acid and AH111501 sodium salt. The mixture will be used to pre-fill a sterile fluid path using laser welding technique under aseptic conditions. The pre-filled sterile fluid path will

be stored frozen at <-20 degrees Celsius until the time of clinical use in the Surbeck imaging lab [redacted] on the Mission Bay campus. At the time of clinical use, the sterile fluid path will be thawed using a warming module housed within the SPINLab™ instrument.

The hyperpolarization process will utilize the automated GE SPINLab™ hyperpolarizer, which is situated in a room adjacent to the MR suite. The mixture of ^{13}C-pyruvic acid and AH111501 sodium salt will be hyperpolarized by DNP at low temperature (<1 degree K) using a 5T magnet and zero-helium loss cryogenics system. Following hyperpolarization, the IMP will be dissolved in sterile fluid water, filtered to remove AH111501 to a level below 3 μM, diluted and neutralized with TRIS/EDTA buffered hydroxide solution, all in an automated fashion using the pre-fabricated sterile fluid path and SPINLab™ system. An integrated quality control (QC) system will analyze the IMP for pH, temperature, concentration, and polarization level, before it is approved for use by the pharmacist. A sample of IMP will be collected for sterility testing. The final IMP is automatically collected within a syringe. The yield is expected to be ~40 mL of 250 mM ^{13}C-pyruvate. Following approval for use from the pharmacist, the syringe containing IMP is delivered to the MRI scanning room and connected to the tubing connected to the infusion pump. The appropriate injection volume is then delivered via the infusion pump to the subject at a rate of 5 ml/second. Note that for different shipments of ^{13}C-pyruvate, the concentration may be slightly different than 250 mM. If a slightly different concentration of ^{13}C-pyruvate is available, the appropriate injection volume will be different but the amount of ^{13}C-pyruvate injected will be unchanged. If this course of action is taken, the updated injection volume will be placed in each patient's chart so that it is clear exactly what concentration and volume the patient received.

The IMP is a colorless to slightly colored, clear liquid.

Warning: Do not use the IMP if any particulates are visible in the solution.

Disposal: Unused substances can be disposed of by flushing down a normal sink using tap water.

Safety Information

Earlier studies of nonhyperpolarized pyruvate outside of the United States were summarized in the background section.

Reported IMP-related AEs in previous studies in humans include:

Cardiovascular: dizziness, catheter site hematoma, heart rate increased, hypertension

Constitutional symptoms: hypoesthesia, unresponsive to stimuli, feeling hot/flushing, fatigue, feeling abnormal

Gastrointestinal: dysgeusia, dry mouth

Genitourinary: micturition urgency

Pain: catheter site pain

Pulmonary: pharyngolaryngeal pain

Neurologic: headache (migraine), parosmia

However, the quality of these prior studies was unclear. The toxicity of the phase I study, which included comprehensive safety monitoring, is summarized in the Background section.

The dosage of IMP that will be used in this study, and the injection rate of 5 ml/second, have been shown to be safe and well tolerated in the phase I study completed at UCSF. Safety monitoring included continuous lead 2 EKG monitoring for 10 minutes after injection, monitoring of vital signs, injection site, EKG, and adverse events, and laboratories for two hours after injection. Patients were also evaluated clinically 24 hours and 7 days after injection. No safety concerns were observed.

4.2 Drug Accountability

The Investigational Pharmacist will manage drug accountability records.

4.3 Drug Ordering

UCSF will obtain supplies for IMP compounding directly from the manufacturer as study supply.

5 Study Procedures and Observations

Schedule of Procedures and Observations

A written, signed, informed consent form (ICF) and a Health Insurance Portability and Accountability Act (HIPAA) authorization must be obtained before any study-specific assessments are initiated. A copy of the signed ICF will be given to the subject and a copy will be filed in the medical record. The original will be kept on file with the study records.

All patients who are consented will be registered in OnCore®, the UCSF Helen Diller Family Comprehensive Cancer Center Clinical Trial Management System (CTMS). The system is password protected and meets HIPAA requirements.

A list of study subjects will be completed, and will include each subject's study number and initials. The investigator must also maintain a separate log of all the subjects screened for participation in the study but who will not participate, the reasons for their exclusion or non-participation, their initials, and the date on which the subject was excluded.

5.1 Screening Assessments

- The Screening procedures and assessments must be completed within 28 days of baseline MR scan except for staging scans which need to be completed within 12 weeks of baseline MR.
- Physical examination - This will include assessment for the presence of abnormalities in general appearance, lungs, cardiovascular system, and abdomen.
- Vital signs including heart rate and blood pressure
- Complete medical history and demographic information
- Baseline conditions assessment
- Concomitant medications
- Disease assessment
- Performance status
- Laboratory procedures
    - Complete blood count (CBC) with differential
    - Blood chemistry assessment including creatinine, AST, ALT, total bilirubin, and lactate dehydrogenase (LDH)
- Electrocardiogram (ECG)
- Staging scans cross-sectional imaging of the abdomen/pelvis (CT or MRI; with IV contrast if medically permissible) (within 3 months of baseline MR scan) (chest imaging is optional)
- **Selection of target lesion:** Study investigators will review the results of standard scans to select the target lesion amenable to metabolic imaging with HP C-13 pyruvate and paired tumor biopsy (review for biopsy in Part B only).

5.2 Metabolic MR Imaging

Pre-Scan Evaluation

Vital signs (systolic and diastolic blood pressure, heart rate) will be monitored at screening and baseline (immediately before the subject is transferred to the MR suite).

Before vital signs are measured, the subject should be resting for at least 5 minutes (if possible). The same position will be used each time vital signs are measured for a given subject and blood pressure will be measured from the arm contra-lateral to the site of IMP administration whenever possible.

Standard MR scanning

Patient is transferred to the MR suite. High spatial resolution MRI to visualize the target lesion using appropriate radiofrequency coil will be performed with axial and coronal T1 and T2 ^{1}H fast spin echo MRI. Gadolinium contrast will be administered intravenously and contrast-enhanced images will be obtained.

Hyperpolarized C-13 Injection and Dynamic ^{13}C MR imaging

Next, hyperpolarized C-13 pyruvate will be injected intravenously at a rate of 5 mL/second followed by a 20 mL saline flush at 5 mL/second.

1-2 minutes post-injection

- Dynamic ^{13}C MRI
    - ^{13}C 3D MRSI sequence with Multiband spectral-spatial excitation pulse for minimal [1-^{13}C]pyruvate saturation and echo-planar spectroscopic imaging (EPSI) readout for accelerated spectral-spatial sampling.
    - The pre-selected target lesion will be imaged on a GE 5T system with parameters typically being T_E = 3ms, T_R = 125ms, 10 Hz spectral resolution, 581 Hz spectra bandwidth, 8 (PE) x 18 (EPSI) matrix, 5x5 mm resolution, 5mm slice thickness, and 5 s per image. The sequence will be started 5 sec after completing the injection (including catheter flushing) of 250mM HP pyruvate (dose = 0.43 mL/kg, 20 to 45 cc's).
    - A "clamshell" volume ^{13}C transmit and/or external RF coils will be used for signal excitation and reception.
    - A second hyperpolarized C-13 injection and dynamic 13-C MRI scan may be performed within 15 to 60 minutes following completion of first scan.. This repeat injection and scan is optional.

Post-injection safety monitoring:

Vital signs (blood pressure and heart rate), adverse event assessment, and injection site monitoring will be performed at 30 minutes post-injection.

Reporting of MR Findings:

The HP C-13 pyruvate/MR scan is considered a research test and as such, will not be used in the clinical management of patients enrolled on the study. A trained radiologist will review all images within 24 hours of scan completion. If there are any unexpected clinically relevant

abnormal findings, these results will be communicated to patient and treating health care provider with 24 hours of completion of MR scan.

5.3 Tumor Biopsy (Part B only; optional)

Patients enrolled in Part B will undergo an optional core needle tumor biopsy of the pre-selected target lesion following completion of baseline metabolic MR scan but prior to initiation of PI3K/mTOR pathway inhibitor. The fresh biopsy sample will be delivered to the Munster lab and will be analyzed for markers of PI3K/mTOR pathway activation, including phosphorylated S6 and Akt, as well as markers of glycolytic activity including LDH expression. When sufficient tissue is available, expression level of MCT1 and MCT 4 (major lactate transporters) will be measured

5.4 Initiation of PI3K/mTOR Pathway-Directed Therapy (Part B Only)

Patients enrolled in Part B will subsequently begin treatment with systemic therapy directed towards the PI3K/mTOR pathway following completion of baseline/screening procedures. Treatment may be given as standard of care with commercial supply (e.g. everolimus) or via participation on therapeutic clinical trial. The study procedures for patients enrolled on a therapeutic trial take precedent over the procedures outlined in the current protocol.

5.5 Day 21 (+/- 14 day) Metabolic MR/HP C-13 Pyruvate study (Part B Only)

Patients will undergo repeat MR scan as per above procedures after 21 days (+/- 14 days) of treatment with androgen signaling inhibitor. Where applicable, patients should take the PI3K/mTOR pathway inhibitor on the day of the MR scan. The target lesion to be evaluated by metabolic MRI will be the same as the prior MR unless lesion has become inevaluable in the interim in the judgment of study investigators. In these cases, an alternate lesion (evaluable baseline MR whenever possible, and adjacent to original target lesion) will be selected for analysis.

5.6 Repeat Tumor Biopsy (Part B Only; Optional)

Patients will undergo optional repeat core needle tumor biopsy following completion of the second MR scan. When possible, patients should remain on PI3K/mTOR pathway inhibitor through the time of the follow up biopsy. The same target lesion will be selected as that biopsied at baseline prior to study initiation whenever possible. In the event that the original target lesion is no longer accessible for repeat tumor biopsy, an alternative metastatic lesion will be selected. Whenever possible, this alternate lesion should be evaluable by metabolic MR imaging.

5.7 Every 6-12 weeks (Part B Only)

Patients will undergo standard disease assessments including standard staging scan every 6-12 weeks per standard of care or per protocol on therapeutic clinical trial. Patients will be followed discontinuation of PI3K/mTOR pathway inhibitor therapy, due to disease progression, unacceptable toxicity, or patient/physician decision to discontinue therapy.

Table 2: Schedule of Study Procedures and Assessments

Period/ Procedure	Screening (28 Days)	Cycle 1 Day 1	Day 21 (+/- 14 days)	Day 28 (+/- 14 days)	Every 6-12 Weeks	Disease Progression
Informed consent	X					
Baseline conditions	X					
AE assessment	X		X			
Concomitant medications	X		X			
Physical exam	X					
Vital signs	X		X			
Medical history/ Demographics	X					
Tumor Assessment by Standard Scans (within 3 months of C1D1)	X				X (Part B only)	
Performance status	X					
CBC w/ Diff	X					
Blood chemistry	X					
HP C-13/metabolic MRI	X		X (Part B only)			
ECG/EKG	X					
Part B Only						
PI3K/mTOR Pathway Inhibitor		→	→	→	→	→
Tumor biopsy (Optional)	X			X		

5.8 Supportive Care and Concomitant Medications

There are no prohibited medications with hyperpolarized C-13 pyruvate administration.

Subjects should receive full supportive care as medically indicated.

The Principal Investigator or designee will be present during the administration and monitoring period.

Any events occurring during or subsequent to the administration of hyperpolarized C-13 pyruvate will be addressed as required by the monitoring nurse and/or physician as deemed appropriate.

Additional toxicities that arise, including the determination that the injection administered was not sterile, will be treated at the discretion of the treating physician.

A site-specific, radiology tackle-box will be present at the imaging site. This tackle-box contains at a minimum: epinephrine 1mg/1ml, diphenhydramine 50 mg/ml, glucose tablets, NaCl 0.9% 500 ml bag, atropine 1mg/ml, methylprednisolone 125mg/2ml vial, phentolamine 5 mg vial, albuterol 5.5 gram inhaler, sterile water 10ml vial.

6 Reporting and Documentation of Results

6.1 Evaluation of Safety

The study will use the CTCAE v4.0 for reporting of adverse events that occur within 3 days of each imaging procedure and any biopsy-related adverse events.

6.2 Definitions of Adverse Events

6.2.1 Adverse Event

An adverse event (also known as an adverse experience) is defined as any untoward medical occurrence associated with the procedure, whether or not considered related. More specifically, an adverse event (can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the procedure, without any judgment about causality. An adverse event can arise from any use of the procedure.

6.2.2 Adverse reaction

An adverse reaction is defined as any adverse event caused by the procedure. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the procedure caused the event.

6.2.2.1 Suspected

A suspected adverse reaction is defined as any adverse event for which there is a reasonable possibility that the procedure caused the adverse event. For the purposes of IND safety reporting, “reasonable possibility” indicates that there is evidence to suggest a causal relationship between the procedure and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

6.2.2.2 Unexpected

An adverse event or suspected adverse reaction is considered *unexpected* if it is not not consistent with the risk information described in the general investigational plan or elsewhere in the protocol. Adverse events that would be anticipated to occur as part of the disease process are considered *unexpected* for the purposes of reporting because they would not be listed in the protocol as being related to the imaging procedure.

6.2.2.3 Serious

An adverse event or suspected adverse reaction is considered *serious* if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- Life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life function
- Congenital anomaly/birth defect

Important medical events that may not result in death, are life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to

prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

6.2.2.4 Life-threatening

An adverse event or suspected adverse reaction is considered *life-threatening* if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

6.3 Recording of an Adverse Event

All grade 3 and above adverse events will be recorded using the NCI CTCAE v4.0. The Investigator will assign attribution of the possible association of the event with the imaging procedure.

Relationship	Attribution	Description
Unrelated to investigational intervention	Unrelated	The AE *is clearly NOT related* to the intervention
	Unlikely	The AE *is doubtfully related* to the intervention
Related to investigational intervention	Possible	The AE *may be related* to the intervention
	Probable	The AE *is likely related* to the intervention
	Definite	The AE *is clearly related* to the intervention

Signs or symptoms reported as adverse events will be graded and recorded by the Investigator according to the CTCAE. When specific adverse events are not listed in the CTCAE they will be graded by the Investigator as *none*, *mild*, *moderate* or *severe* according to the following grades and definitions:

Grade 0 No AE (or within normal limits)

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated

Grade 2 Moderate; minimal, local, or noninvasive intervention (e.g., packing, cautery) indicated; limiting age-appropriate instrumental activities of daily living (ADL)

Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL

Grade 4: Life-threatening consequences; urgent intervention indicated

Grade 5: Death related to AE

6.4 Follow-up of Adverse Events

All adverse events will be followed with appropriate medical management until resolved. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. For selected adverse events for which the imaging procedure was stopped, a second attempt at imaging may be conducted if considered both safe and ethical by the Investigator.

Reporting to the Data and Safety Monitoring Committee

If a death occurs during the treatment phase of the study or within 30 days after the imaging procedure and it is determined to be related either to the study procedure, the Investigator or his/her designee must notify the DSMC Chair (or qualified alternate) within 1 business day of knowledge of the event. The contact may be by phone or e-mail.

Reporting to UCSF Institutional Review Board (IRB)

The Principal Investigator must report events meeting the UCSF IRB definition of “Unanticipated Problem” (UP) within 5 business days of his/her awareness of the event.

7 Statistical Considerations and Image Analysis

7.1 Image Processing and Analysis

For each patient, a target metastatic lesion will be selected on the basis of standard imaging (CT or MRI). A region-of-interest (ROI) will be manually drawn in the target lesion, and the same lesion will be evaluated on serial MR scans if the lesion continues to remain evaluable by MRI. If lesion is no longer evaluable on follow up scan, an alternative lesion will be selected on the basis of proximity to original lesion and accessibility by radiofrequency coil for purposes of metabolic MR evaluation.

For the analysis and interpretation of the HP ^{13}C MR imaging data, we will utilize the open-source DICOM software package (SIVIC) to align, display and quantitatively interrogate serial multiparametric imaging data. Intra-tumoral ROI will be used to quantify peak HP lactate/pyruvate ratio values in the selected volumes of interest.

In an exploratory fashion, additional metastatic sites in addition to the target lesion will be analyzed and interpreted to determine intra-tumoral peak HP lactate/pyruvate ratio.

7.2 Study Endpoints

Primary endpoints:

Part A: Signal-to-noise ratio with respect to intra-tumoral hyperpolarized C-13 lac/pyr ratio detected within target liver or other intraabdominal metastasis in patients with advanced solid tumor malignancies.

Part B: Mean percent change from baseline in peak intra-tumoral hyperpolarized lactate/pyruvate ratio after initiation of treatment with PI3K/mTOR pathway inhibitor.

Secondary endpoints

- Frequency of adverse events as graded by Common Toxicity Criteria version 4.0
- Association between percent change from baseline in peak intra-tumoral HP lactate/pyruvate ratio on metabolic MR imaging with subsequent outcomes on PI3K/mTOR pathway inhibitor therapy including clinical benefit rate and progression-free survival (Part B)
- Intra-patient reproducibility of HP lac/pyr ratio for patients who undergo repeated dose imaging studies, as descriptively reported using summary statistics (mean difference, standard deviation, range).

Exploratory endpoint

Association between HP peak lactate/pyruvate signal with immunohistochemical expression level of markers of PI3K/mTOR and glycolytic pathway, including LDH, phosphorylated S6, and phosphorylated Akt. (Part B)

7.3 Sample Size Determination

In Part A of the study, 50 subjects with advanced solid tumor malignancies and at least one metastatic liver or intra-abdominal lesion will undergo hyperpolarized C-13

pyruvate/metabolic MR imaging. The coil arrays will undergo iterative adjustment in geometry and design to optimize the signal-to-noise ratio of intra-tumoral HP lactate/pyruvate.

In Part B of the study, the sample size of 30 evaluable patients is based upon the primary endpoint of this portion of the study, the mean percent change from baseline in peak intra-tumoral lactate/pyruvate ratio in the target metastatic lesion after initiation of treatment with PI3K/mTOR pathway inhibitor. Assuming the intra-tumoral lactate/pyruvate ratio is 0.80 at baseline and the expected ratio at day 14 is 0.45 (e.g. a 35% decline), based on prior pre-clinical studies, then a sample size 30 will have > 85% power to detect the significance of the change in signal under paired t-tests with type I error of 5%.

7.4 Subject Characteristics

Demographic information (e.g. age, race, height, weight, and body mass index) will be summarized using descriptive statistics.

Concurrent medications will be recorded and coded using a standard classification system.

Disease factors for subjects accrued to the study will be characterized using descriptive statistics.

7.5 Replacement Policy

Patients may be replaced in Part B for the following reasons:

- Inability to tolerate/complete imaging scan (with no administration of HP C-13 pyruvate)
- Failure of the HP C-13 pyruvate to pass quality control testing, preventing its administration
- Patients who do not undergo subsequent PI3K/mTOR pathway therapy

7.6 Accrual estimates

The study is estimated to accrue approximately 1-2 patients per month leading to an approximate total accrual period of 24 months.

7.7 Interim Analysis

Interim analyses will be conducted for both safety and feasibility. Toxicity will be reviewed on a continuous basis. If at any time (after two or more patients have been accrued) ≥33% of patients experience ≥grade 2 toxicity (excluding asymptomatic laboratory abnormalities deemed to be clinically insignificant by the PI or designee), accrual will be halted for further evaluation.

At the time that it can be determined that continued accrual does not pose significant safety risks to patients accrual may be re-initiated.

Interim analysis for feasibility in Part B will be performed after 33% of patients have been enrolled. Images will be qualitatively reviewed and preliminary data with respect to signal-to-noise ratio within metastatic lesions will be assessed. If it is determined that insufficient signal-to-noise ratio is present to permit analyses of the primary and secondary endpoints, then study accrual will be halted until proper technological adjustments (e.g. alteration of radiofrequency coils, timing from polarization to injection of tracer) have been implemented to ensure higher quality SNR.

7.8 Analytic Plan

7.8.1 Analysis Population

All data from all subjects dosed in the study will be included in all listings, plots, summary tables, and statistical analyses when appropriate. Missing values will not be substituted by estimated values, but treated as missing in the statistical evaluation.

7.8.2 Primary Analysis

Descriptive statistics will be used to characterize the mean change from baseline in intra-tumoral HP pyruvate/lactate ratio for the study cohort, along with 95% confidence interval.

7.8.3 Analysis of Secondary and Exploratory Endpoints

For the purposes of analyzing the association between percent change from baseline in intra-tumoral peak lactate/pyruvate ratio on HP MRI with subsequent clinical outcomes, the cohort will be dichotomized by the median percent change. The objective response rate by RECIST 1.1 criteria and clinical benefit rate (response or stable disease for > 24 weeks) will be compared between dichotomized groups using the chi-squared test. The log rank test will be used to compare the radiographic progression-free survival between the two groups.

Safety analyses will be performed for all patients having received a dose of HP C-13 pyruvate. The study will use the NCI CTCAE v4.0.

Analyses will be performed to compare the HP MR markers with immunohistochemical and enzyme expression and activity data from biopsy tissue to investigate the molecular and physiological underpinnings of the HP ^{13}C MR data. When both the HP biomarker and molecular biomarker (LDHA activity) are continuous variables, Spearman's rank correlation coefficient will be used to investigate a possible correlation between them. If the outcome is categorical (e.g. immunohistochemistry score 0-1+ vs 2-3+ for pS6, pAkt, LDHA, and MCT 1/4 expression), the HP biomarker distributions will be compared between the two categories using the Mann-Whitney test.

Intra-patient reproducibility of HP lac/pyr ratio for patients who undergo repeated dose imaging studies will be descriptively reported using summary statistics (mean difference, standard deviation, range).

No adjustment will be made for multiple comparisons for the analysis of the secondary and exploratory endpoints.

8 Study Management

8.1 Pre-study Documentation

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki as stated in 21 CFR §312.120(c)(4); consistent with GCP and all applicable regulatory requirements.

Before initiating this trial, the Investigator will have written and dated approval from the Institutional Review Board for the protocol, written informed consent form, subject recruitment materials, and any other written information to be provided to subjects before any protocol related procedures are performed on any subjects.

8.2 Institutional Review Board Approval

The protocol, the proposed informed consent form, and all forms of participant information related to the study (e.g. advertisements used to recruit participants) will be reviewed and approved by the UCSF Institutional Review Board (IRB). Prior to obtaining IRB approval, the protocol must be approved by the Helen Diller Family Comprehensive Cancer Center Site Committee and by the Protocol Review Committee (PRC). The initial protocol and all protocol amendments must be approved by the IRB prior to implementation.

8.3 Informed Consent

All participants must be provided a consent form describing the study with sufficient information for each participant to make an informed decision regarding their participation. Participants must sign the CHR-approved informed consent form prior to participation in any study specific procedure. The participant must receive a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

8.4 Changes in the Protocol

Once the protocol has been approved by the UCSF IRB, any changes to the protocol must be documented in the form of an amendment. The amendment must be signed by the Investigator and approved by PRC and the IRB prior to implementation.

If it becomes necessary to alter the protocol to eliminate an immediate hazard to patients, an amendment may be implemented prior to IRB approval. In this circumstance, however, the Investigator must then notify the IRB in writing within five (5) working days after implementation. The Study Chair and the UCSF study team will be responsible for updating any participating sites.

8.5 Handling and Documentation of Clinical Supplies

The UCSF Principal Investigator and each participating site will maintain complete records showing the receipt, dispensation, return, or other disposition of all investigational drugs. The date, quantity and batch or code number of the drug, and the identification of patients to whom IMP has been dispensed by patient number and initials will be included. The sponsor-investigator will maintain written records of any disposition of the IMP.

The Principal Investigator shall not make the investigational drug available to any individuals other than to qualified study patients. Furthermore, the Principal Investigator will not allow the investigational drug to be used in any manner other than that specified in this protocol.

8.6 Case Report Forms (CRFs)

The Principal Investigator and/or his/her designee, will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study. Study specific Case Report Forms (CRFs) will document safety and treatment outcomes for safety monitoring and data analysis. All study data will be entered into OnCore® via standardized CRFs in accordance with the CTMS study calendar, using single data entry with a secure access account. The Clinical Research Coordinator (CRC) will complete the CRFs as soon as possible upon completion of the study visit; the Investigator will review and approve the completed CRFs.

The information collected on CRFs shall be identical to that appearing in original source documents. Source documents will be found in the patient's medical records maintained by UCSF personnel. All source documentation should be kept in separate research folders for each patient.

In accordance with federal regulations, the Investigator is responsible for the accuracy and authenticity of all clinical and laboratory data entered onto CRFs. The PI will approve all completed CRFs to attest that the information contained on the CRFs is true and accurate.

All source documentation and CTMS data will be available for review/monitoring by the UCSF DSMC and regulatory agencies.

The Principal Investigator will be responsible for ensuring the accurate capture of study data. At study completion, when the CRFs have been declared to be complete and accurate, the database will be locked. Any changes to the data entered into the CRFs after that time can only be made by joint written agreement among the Study Chair, the Trial Statistician, and the Protocol Project Manager.

8.7 Oversight and Monitoring Plan

The UCSF Helen Diller Family Comprehensive Cancer Center DSMC will be the monitoring entity for this study. The UCSF DSMC will monitor the study in accordance with the NCI-approved Data and Safety Monitoring Plan (DSMP). The DSMC will routinely review all adverse events and suspected adverse reactions considered "serious". The DSMC will audit study-related activities to ensure that the study is conducted in accordance with the protocol, local standard operating procedures, FDA regulations, and Good Clinical Practice (GCP). Significant results of the DSMC audit will be communicated to the IRB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as applicable.

8.8 Record Keeping and Record Retention

The Principal Investigator is required to maintain adequate records of the disposition of the drug, including dates, quantity, and use by subjects, as well as written records of the disposition of the drug when the study ends.

The Principal Investigator is required to prepare and maintain adequate and accurate case histories that record all observations and other data pertinent to the investigation on each individual administered the investigational drug or employed as a control in the investigation. Case histories include the case report forms and supporting data including, for example, signed and dated consent forms and medical records including, for example, progress notes of the physician, the individual's hospital chart(s), and the nurses' notes. The case history for each individual shall document that informed consent was obtained prior to participation in the study.

Study documentation includes all CRFs, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

In accordance with FDA regulations, the investigator shall retain records for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified.

References

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Appendix 1 Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity Fully active, able to carry on all pre-disease performance without restriction	100	Normal, no complaints, no evidence of disease
		90	Able to carry on normal activity; minor signs or symptoms of disease
1	Symptoms, but ambulatory Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work)	80	Normal activity with effort; some signs or symptoms of disease
		70	Cares for self, unable to carry on normal activity or to do active work
2	In bed < 50% of the time Ambulatory and capable of all self-care, but unable to carry out any work activities Up and about more than 50% of waking hours	60	Requires occasional assistance, but is able to care for most of his/her needs
		50	Requires considerable assistance and frequent medical care
3	In bed > 50% of the time Capable of only limited self-care, confined to bed or chair more than 50% of waking hours	40	Disabled, requires special care and assistance
		30	Severely disabled, hospitalization indicated Death not imminent
4	100% bedridden Completely disabled Cannot carry on any self-care Totally confined to bed or chair	20	Very sick, hospitalization indicated Death not imminent
		10	Moribund, fatal processes progressing rapidly
5	Dead	0	Dead

Appendix 2 Data and Safety Monitoring Plan for a Phase 2 or 3 Institutional Study

The UCSF Helen Diller Family Comprehensive Cancer Center (HDFCCC) Data and Safety Monitoring Committee (DSMC) is responsible for monitoring data quality and subject safety for all HDFCCC institutional clinical studies. A summary of DSMC activities for this study include:

- Review of subject data
- Review of suspected adverse reactions considered "serious"
- Monitoring every six months (depending on study accrual)
- Minimum of a yearly regulatory audit

Monitoring and Reporting Guidelines

Investigators will conduct continuous review of data and subject safety and discuss each subject's treatment at monthly Site Committee meetings. These discussions are documented in the Site Committee meeting minutes. The discussion will include the number of subjects, significant toxicities in accordance with the protocol, and observed responses.

All institutional Phase 2 or 3 studies are designated with a moderate risk assessment. The data is monitored twice per year with twenty percent of the subjects monitored (or at least three subjects if the calculated value is less than three).

Adverse Event Review and Monitoring

All grade(s) 3-5 adverse events, whether or not unexpected, and whether or not considered to be associated with the use of the study drug, will be entered into OnCore®, UCSF's Clinical Trial Management System.

All grade(s) 3-5 adverse events entered into OnCore® will be reviewed on a monthly basis at the Site Committee meetings. The Site Committee will review and discuss the selected toxicity, the toxicity grade, and the attribution of relationship of the adverse event to the administration of the study drug(s).

In addition, all suspected adverse reactions considered "serious" entered into OnCore®, will be reviewed and monitored by the Data and Safety Monitoring Committee on an ongoing basis and discussed at DSMC meetings, which take place every six weeks.

If a death occurs during the treatment phase of the study or within 30 days after the last administration of the study drug(s) and is determined to be related either to the investigational drug or any research related procedure, the Investigator or or the assigned designee must be notified within 1 business day from the participating site(s) and the Study Chair must then notify the DSMC Chair or qualified alternate within 1 business day of knowledge of this event. The contact may be by phone or e-mail.

Increase in Adverse Event Rates

If an increase in the frequency of Grade 3 or 4 adverse events (above the rate reported in the Investigator Brochure or package insert) is noted in the study, a report should be submitted to the DSMC at the time the increased rate is identified. The report will indicate if the incidence of adverse events observed in the study is above the range stated in the Investigator Brochure or package insert.

If at any time the Study Chair stops enrollment or stops the study due to safety issues, the DSMC Chair and DSMC Manager must be notified within 1 business day via e-mail. The DSMC must receive a formal letter within 10 business days and the IRB must be notified.

Data and Safety Monitoring Committee Contacts

DSMC Chair: [redacted]
Phone: [redacted]
Email: [redacted]
Address: Box 1705
UCSF
San Francisco, CA 94158

DSMC Monitors
Box 0128
UCSF Helen Diller Family
Comprehensive Cancer Center
San Francisco, CA 94143

* DSMP approved by NCI 09/February2012

Appendix 3 UCSF Policy/Procedure for Required Regulatory Documents for UCSF Investigator-Initiated Oncology Clinical Trials with an Investigator held Investigational New Drug (IND)

Purpose

This policy defines the required Regulatory Documents for Single Site and Multicenter Investigator Initiated Oncology Clinical Trials at the Helen Diller Family Comprehensive Cancer Center (HDFCCC) where the Principal Investigator (PI) holds the IND.

Background

The International Conference on Harmonization (ICH) Good Clinical Practices (GCP) Guidelines define Essential Regulatory Documents as those documents which individually and collectively permit evaluation of the conduct of a trial and the quality of data produced. These documents serve to demonstrate compliance with standards of GCP and with all applicable regulatory requirements. Filing essential documents in a timely manner can greatly assist in the successful management of a clinical trial.

The Regulatory Documents will consist of electronic files in both iMedRIS and OnCore®, as well as paper files in the Regulatory Binders for both the Coordinating Site and the Participating Site(s) in the HDFCCC Investigator Initiated Oncology Clinical Trials.

Procedures

1. HDFCCC Essential Regulatory Documents

Documents Filed in iMedRIS:

- IRB approvals for initial submission of application, all modifications, and continuing annual renewals
- Current and prior approved protocol versions with signed protocol signature page(s)
- Committee for Human Research (IRB) approval letters and Informed Consent Form(s) (ICF)
- Current and prior versions of the Investigator Brochure (IB).
- Serious Adverse Event Reporting
- Protocol Violations and Single Patient Exception (SPE) Reports to IRB with supporting fax documentation

Documents Filed in OnCore®:

- Package Insert (if the study drug is commercial) or Investigator Brochure
- Protocol Review Committee (PRC) approved protocols, protocol amendments and Summary of Changes (SOC)
- Patient handouts
- Screening/enrollment log
- Data and Safety Monitoring Committee (DSMC) monitoring reports
- OnCore® Case Report Form (CRF) completion manual

Documents Filed in Regulatory Binder:

- Completed Food and Drug Administration (FDA) 1572 document with Principal Investigator's signature
- For all Principal Investigators and Sub-Investigators listed on the FDA 1572, will need Financial Disclosure Forms, CVs, MD Licenses, Drug Enforcement Agency (DEA) Licenses, and Staff Training Documents (i.e. Collaborative Institute Training Initiative (CITI), etc.)
- Site Initiation Visit (SIV) minutes and correspondence with participating site(s).
- As applicable, approvals for Biosafety Committee, Radiation Committee, and Infusion Center
- Serious Adverse Event (SAE) reports to IRB and sponsor.
- MedWatch reporting to FDA and sponsor
- Delegation of Authority Form
- Drug Destruction Standard Operating Procedure (SOP)
- For all laboratories listed on the FDA 1572, will need CLIA certifications, CAP certifications, lab licenses, CVs of Lab Directors, and laboratory reference ranges