UCSF Helen Diller Family Comprehensive Cancer Center

Hyperpolarized ^{13}C pyruvate MRI for treatment response assessment in pancreatic ductal adenocarcinoma

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Protocol Signature Page

Protocol No.: CC# 20925

I agree to follow this protocol version as approved by the UCSF Protocol Review Committee (PRC), Institutional Review Board (IRB), and Data and Safety Monitoring Committee (DSMC).

I will conduct the study in accordance with Good Clinical Practices (ICH-GCP) and the applicable IRB, ethical, federal, state, and local regulatory requirements.

I certify that I, and the study staff, have received the required training to conduct this research protocol.

I agree to maintain adequate and accurate records in accordance with IRB policies, federal, state and local laws and regulations.

UCSF Principal Investigator

_____
Printed Name

_____ _____
Signature Date

Abstract

Title	Hyperpolarized ^{13}C pyruvate metabolic MRI for treatment response assessment in pancreatic ductal adenocarcinoma
Study Description	This is a pilot prospective study to investigate the utility of HP 13C pyruvate MRI for assessing tumor metabolism and monitoring early treatment response in patients with locally advanced or metastatic PDA.
Phase of Study	Pilot
Investigational Product	HP ^{13}C pyruvate injection
Study Population	Patients with locally advanced or metastatic pancreatic ductal adenocarcinoma (PDA) will be enrolled in parallel in one of two cohorts: Cohort A (N=20): Patients who are already on therapy will undergo hyperpolarized (HP) ^{13}C pyruvate MRI at a single time-point. Cohort B (N=20): Patients will undergo HP ^{13}C pyruvate MRI at baseline prior to the initiation of a new line of therapy (standard-of-care first- or second-line systemic therapies, radiotherapy, or therapies as part of a clinical trial), and again at 4 weeks (+/- 2 weeks) following treatment initiation.
Primary Objective	Cohort A: To determine the signal-to-noise ratio of 13C pyruvate metabolism measures (peak 13C lactate/pyruvate ratio, 13C lactate/pyruvate area-under-the-curve (AUC) ratio, and apparent rate constant for pyruvate-to-lactate conversion, kPL) in the target tumor (primary tumor and/or abdominal metastases). Cohort B: To determine the percent changes in the target tumor (primary tumor and/or abdominal metastases) 13C pyruvate metabolism measures (peak 13C lactate/pyruvate ratio, 13C lactate/pyruvate AUC ratio, and kPL) between pre-treatment scan and scan obtained at 4-week (+/- 2 weeks) following treatment initiation.
Secondary Objectives	Cohort A and B: To determine the repeatability of 13C pyruvate metabolism measures in the target tumor (primary tumor and/or abdominal metastasis) in patients with same-day repeated dose. Cohort B: To determine whether the baseline or the changes in the target tumor (primary tumor and/or abdominal metastases) 13C pyruvate metabolism measures at 4 weeks (+/-2 weeks) following treatment initiation are associated with the best objective response as defined by RECIST criteria on subsequent clinical CT scans.
Exploratory Objective	Cohort A and B: To explore 13C pyruvate metabolism between the primary tumor and abdominal metastases (when present) both at baseline and following treatment.
Sample Size	40 patients will be enrolled- 20 patients in Cohort A, and 20 patients in Cohort B.
Duration of study	The study is estimated to reach completion approximately 36 months from first patient is enrolled.

List of Abbreviations

AE	adverse event
BUN	blood urea nitrogen
CBC	complete blood cell (count)
CNS	central nervous system
CR	complete response
CRF	case report form
CT	computerized tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTMS	Clinical Trial Management System
DFS	disease-free survival
DLT	dose limiting toxicity
DSMC	Data and Safety Monitoring Committee
DSMP	Data and Safety Monitoring Plan
ECG/EKG	electrocardiogram
ECOG	Eastern Cooperative Oncology Group
FDA	Food and Drug Administration
FDG	Fluorodeoxyglucose
FLC	free light chain
GCP	Good Clinical Practice
GFR	glomerular filtration rate
HDFCCC	Helen Diller Family Comprehensive Cancer Center
HIPAA	Health Insurance Portability and Accountability Act
ICF	informed consent form
ICH	International Conference on Harmonization
IDS	Investigational Drug Services (UCSF)
IND	investigational new drug application
IP	investigational product
IRB	Institutional Review Board
IV	intravenous
LDH	lactate dehydrogenase
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
NCI	National Cancer Institute
ORR	overall response rate
PD	disease progression

List of Abbreviations

PK	pharmacokinetics
PO	*Per os* (by mouth, orally)
PR	partial response
PRC	Protocol Review Committee (UCSF)
SD	stable disease

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1 Introduction

1.1 Experience of ^{13}C pyruvate injection in humans

As a first step in evaluating the use of ^{13}C 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-natC] pyruvate was used. This study was completed in December 2007 (Investigators' Brochure). In the original protocol, it was planned that doses up to 0.71 ml/kg body weight (bw) 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 13C pyruvate injection and 2 received saline. Doses up to 0.43 ml/kg bw were well tolerated and no IMP-related AEs were observed that warranted un-blinding the study to reveal which subjects had received pyruvate Injection versus placebo. However, in the 0.57 ml/kg bw cohort, 2 of 6 subjects showed IMP-related non-serious AEs ("unresponsiveness" [redacted] and "flushing" accompanied by changes in blood pressure and heart rate [redacted]) that the principal investigator considered to be concerning and related to the administration of IMP. The study was un-blinded 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 [redacted] 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 ^{13}C pyruvate Injection was conducted in elderly (60 years of age) volunteers. All doses up to 0.43 ml/kg bw 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.

1.1.1 UCSF Phase I experience of hyperpolarized ^{13}C pyruvate in prostate cancer patients

On the basis that doses of pyruvate injection up to 0.43 ml/kg bw 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 (IND # 109956). In this study, three dose levels of HP ^{13}C pyruvate injection, 0.14 ml/kg bw, 0.28 mg/kg bw, and 0.43 ml/kg bw were evaluated. Thirty-one patients ([redacted]) 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:

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 [redacted], 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).

Intensive monitoring was performed 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.

Since the initial UCSF Phase I study, multiple other human studies with hyperpolarized ^{13}C pyruvate MRI in patients with various diseases as well as in normal healthy volunteers have been conducted(1-5), none have reported any safety concerns.

1.2 Safety of repeated HP ^{13}C 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. 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 (6). Moreover, two injections of hyperpolarized [1-^{13}C]pyruvate have already been approved and performed in over 20 human studies at UCSF (CHR#13-12719, CHR 17-24246). These studies have demonstrated the safety of serial injections without any adverse effects noted.

1.3 Rationale for use of HP ^{13}C Pyruvate in patients with pancreatic cancer

Need for rapid response monitoring in PDA: Pancreatic ductal adenocarcinoma (PDA) is the 3rd leading cause of cancer related death in the US and is anticipated to become the 2nd by 2030(7). The majority of PDA patients (> 75%) present with locally advanced or metastatic disease for which systemic chemotherapy is the only life-prolonging treatment (8). The length of time these patients spend on effective therapy is the most important factor in their survival. Therefore, there is an urgent need for biomarkers to accurately assess treatment response, and to do so early so that patients can be spared the significant toxicities of ongoing ineffective treatment and can instead be offered alternative treatment (i.e. combination chemotherapy of a differing backbone from that administered in the first line) with a better chance of efficacy. Additionally, we need alternative endpoints for clinical trials other than overall survival, which is often complex in PDA and is often a function of treatment efficacy coupled with performance status and other factors. *Given the deadliness of this disease, we need to rapidly determine response in order to inform decision of whether to continue an effective treatment or discontinue an ineffective one (9).*

Limitations of current response assessment tools in PDA: Objective treatment response in PDA is commonly assessed by measuring changes in tumor size at anatomic imaging according to RECIST (Response Evaluation Criteria in Solid Tumors) criteria several months into treatment. However, a change in tumor size is a *relatively late* endpoint in PDA. In addition, monitoring response to therapy in the primary tumor is particularly challenging because of the extensive

desmoplasia and inflammation associated with the tumor and the difficulty in defining tumor margin (10). As a result, many patients' functional status declines while we wait to determine whether or not the treatment is working at imaging read-out points (typically 8-12 weeks apart following treatment initiation). *Many of these patients progress after months of ineffective therapy and are no longer suitable for second line therapy nor eligible for second line trials, resulting in worse survival*.

CA19-9 is a widely used serum marker in PDA, but also has limitations. It requires the presence of the Lewis blood group antigen (a glycosyl transferase) to be expressed, and about 10%-25% of PDA patients have tumors that do not express CA19-9 (11, 12). Additionally, while CA19-9 response over months correlates well with overall survival (13), due to its long half-life, it is not a useful early predictor of response to therapy. In fact, CA19-9 at times can initially INCREASE in patients who will ultimately respond (14). Therefore, while CA19-9 is a useful long-term read-out of response, it is worthless in patients who do not express it, and is not a reliable early predictor of response in those who do.

HP ^{13}C pyruvate MRI: Hyperpolarized (HP) ^{13}C MRI is an emerging molecular imaging method that allows rapid, noninvasive, pathway-specific investigation of dynamic metabolic processes that were previously inaccessible by imaging. Hyperpolarization, achieved through the dynamic nuclear polarization (DNP) technique(15), provides unprecedented gains in sensitivity (> 10,000-fold signal increase) for imaging ^{13}C-labeled bio-molecules that are *endogenous, nontoxic, and nonradioactive*. HP ^{13}C pyruvate is the most widely used and a highly biologically relevant probe, as pyruvate lies at a critical branch point of multiple metabolic pathways, including glycolysis, tricarboxylic acid (TCA) cycle, and amino acid biosynthesis. HP ^{13}C pyruvate MRI has already shown considerable promise in tumor grading (32) and response assessment in numerous preclinical studies(16-27). For example, decreased HP ^{13}C pyruvate to lactate conversion was observed by 24 hours after the initiation of cytotoxic chemotherapy in a lymphoma model(16). In a Myc oncogene driven liver cancer model, increased HP ^{13}C pyruvate conversion to lactate and alanine was observed to precede tumor formation, and there was a dramatic reversal of pyruvate to lactate conversion during early tumor regression before any size change(26).

Notably, while 18-fluoro-deoxyglucose (FDG) PET, which assesses tissue glucose uptake, has shown great value in oncological imaging, HP ^{13}C pyruvate MRI has several unique advantages including its ability to directly interrogate downstream metabolism that is not accessible by FDG PET(21, 28-30). For example, while prostate cancer has similar FDG avidity to the normal prostate(31), a grade-dependent increase in HP ^{13}C pyruvate to lactate conversion has been observed in a mouse prostate cancer model as well in patient-derived prostate cancer tissues(28, 32), and a marked drop in HP ^{13}C pyruvate to lactate conversion occurs following successful treatment(4) (**Fig 1**). As such, HP ^{13}C pyruvate MRI can provide distinct and complementary information in tumor metabolism and response to therapy.

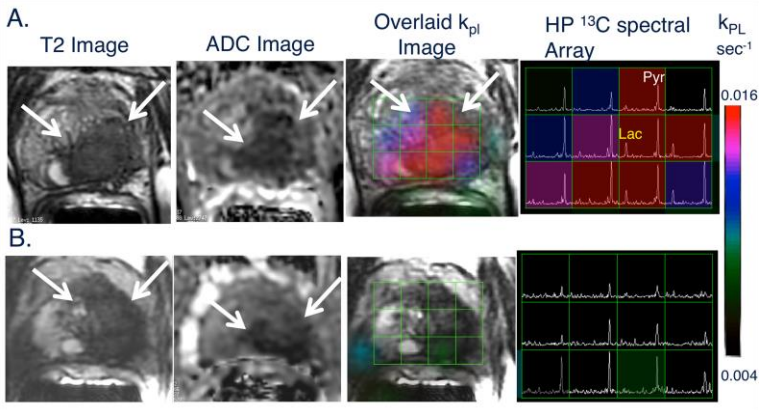

Fig 1. Multi-parametric MRI in a 52-yr-old prostate cancer patient with extensive high-grade (Gleason 4+5) prostate cancer (**A**) before therapy and (**B**) 6 week after initiation of androgen ablation and chemotherapy. Before treatment, the region of prostate cancer can be seen (arrows) as low signal on the T2-weighted and ADC images, and high k_{PL}. At 6 weeks after initiation of androgen deprivation therapy, there was near complete abrogation of elevated HP lactate peaks as seen on the spectral array and marked reduction in tumor k_{PL} (k_{PL} max 0.025 s^{-1} at baseline and 0.007 s^{-1} on follow-up). Notably, there was negligible change in size of tumor on T2-weighted MRI and only a modest change on ADC images, supporting the utility of HP ^{13}C pyruvate MRI to detect early metabolic responses prior to changes on conventional images. Concordant with these findings, the patient subsequently achieved a marked clinical response, with an undetectable serum prostate specific antigen (PSA) nadir at 6 months after treatment initiation.

In the context of PDA, mutations in the KRAS gene (occurring in 90% of PDA) play an important role in metabolic reprogramming(33). In particular, oncogenic KRAS drives glycolytic activity in PDA, with increased expression of several glycolytic enzymes including LDH-A, and elevated levels of glycolysis-associated metabolites including lactate. In transgenic mouse models, HP ^{13}C pyruvate MRI has been shown to detect and monitor the progression from pancreatic intraepithelial neoplasia (PanIN) precursor lesions to PDA, with an increase in ^{13}C lactate/alanine ratio with disease progression(34). Another study in patient-derived mouse xenograft of PDA has demonstrated the ability of HP ^{13}C pyruvate MRI to noninvasively monitor response/resistance to metabolic therapy before tumor size changes (35). A new clinical report has shown the feasibility of acquiring HP ^{13}C pyruvate MRI in PDA patients, with increased ^{13}C lactate/alanine ratio in the primary PDA compared to adjacent pancreas (36). We have also shown the feasibility of HP ^{13}C pyruvate MRI of liver metastases in patients with PDA (**Fig. 2**). The images were acquired using a novel dynamic, metabolite-specific imaging approach that allows for volumetric imaging, and showed a marked increase in the pyruvate to lactate conversion in the liver metastases. Taken together, these findings provide strong rationales supporting the clinical evaluation of this metabolic imaging technology to assess early treatment response/resistance in patients with PDA.

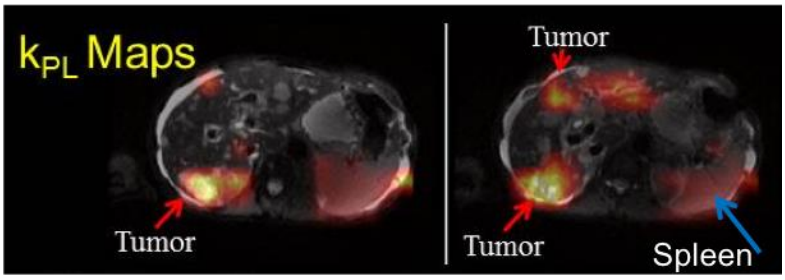

Fig 2. **Quantitative maps of the pyruvate to lactate conversion rate, k_{PL}, in liver metastases from PDA (overlaid on T2 weighted anatomic images)**. Dynamic images of pyruvate and lactate are fit to a kinetic model to extract the first order rate constant (k_{PL}) that characterizes the conversion of pyruvate to lactate, which is elevated in the liver metastases (arrows) in a patient with PDA, reflecting high LDH activity.

2 Study Objectives

2.1 Hypothesis

Our over-arching hypothesis is that early metabolic changes in PDA visualized on HP ^{13}C pyruvate MRI are predictive of treatment response/resistance.

2.2 Primary Objective and Endpoints

Primary Objective	Endpoint(s)	Time Frame
1. To determine the signal-to-noise ratio of ^{13}C pyruvate metabolism (peak ^{13}C lactate/pyruvate ratio, ^{13}C lactate/pyruvate area-under-the-curve (AUC) ratio, and apparent rate constant for pyruvate-to-lactate conversion, k_{PL}) in the target tumor (primary tumor and/or abdominal metastases) in Cohort A.	• signal-to-noise ratio of ^{13}C pyruvate metabolism	From study initiation to enrollment of last subject
2. To determine the percent changes in the target tumor (primary tumor and/or abdominal metastases) ^{13}C pyruvate metabolism (peak ^{13}C lactate/pyruvate ratio, ^{13}C lactate/pyruvate AUC ratio, and k_{PL}) between pre-treatment scan and scan obtained at 4-week (+/-2 weeks) following treatment initiation in Cohort B.	• percent changes in the target tumor ^{13}C pyruvate metabolism	From study initiation to enrollment of last subject

2.3 Secondary Objectives and Endpoints

Secondary Objective	Endpoint(s)	Time Frame
1. To determine the repeatability of ^{13}C pyruvate metabolism measures in the target tumor (primary tumor and/or abdominal metastasis) in patients with same-day repeated dose in Cohort A and B.	• repeatability of ^{13}C pyruvate metabolism measures in the target tumor	From study initiation to enrollment of last subject
2. To determine whether the baseline or the changes in the target tumor (primary tumor and/or abdominal metastases) ^{13}C pyruvate metabolism at 4 weeks following treatment initiation are associated with the best objective response as defined by RECIST criteria on subsequent clinical CT scans in Cohort B.	• The association between the baseline target tumor pyruvate metabolism and tumor best objective response as defined according to RECIST criteria on subsequent standard-of-care CT. • The association between the changes in the target tumor pyruvate metabolism post treatment initiation and tumor best objective response as defined according to RECIST criteria on subsequent standard-of-care CT.	From study initiation to up to 18 months following enrollment of last subject

2.4 Exploratory Objective and Endpoints

To explore ^{13}C pyruvate metabolism between the primary tumor and abdominal metastases (when present) both at baseline and following treatment in both Cohort A and B.

2.5 Imaging Endpoints

Tumor HP ^{13}C pyruvate metabolism (peak lactate/pyruvate ratio, lactate /pyruvate AUC ratio, kPL) will be measured for each patient.

3 Study Design

3.1 Characteristics

This is a pilot prospective study to investigate the utility of HP ^{13}C pyruvate MRI for assessing metabolism and monitoring early treatment response in patients with locally advanced or metastatic PDA. Patients will be enrolled in parallel in one of two cohorts:

Cohort A (N=20): Patients with locally advanced or metastatic PDA who are already on therapy will undergo hyperpolarized (HP) ^{13}C pyruvate MRI at a single time-point.

Cohort B (N=20): Patients with locally advanced or metastatic PDA will undergo HP ^{13}C pyruvate MRI at baseline prior to the initiation of a new line of therapy (standard-of-care first- or

second-line systemic therapies, radiotherapy, or therapies as part of a clinical trial), and again at 4 weeks (+/- 2 weeks) following treatment initiation.

In both Cohort A and B, patients may undergo an optional second dose of ^{13}C pyruvate injection followed by MRI during the same imaging session.

3.2 Sample Size

A total of 40 patients will be enrolled.

3.3 Replacement Policy

Patients may be replaced for the following reasons:

- Inability to tolerate/complete imaging scan (with no administration of HP ^{13}C pyruvate)
- Failure of the HP ^{13}C pyruvate to pass testing, preventing its administration
- Patients who do not subsequently undergo treatment following the baseline HP ^{13}C pyruvate MRI

3.4 Eligibility Criteria

Patients must have baseline evaluations performed within 4 weeks prior to the HP ^{13}C pyruvate MRI and must meet all inclusion and exclusion criteria. In addition, the patient must be thoroughly informed about all aspects of the study, including the study procedures, and required evaluations and all regulatory requirements for informed consent. The written informed consent must be obtained from the patient prior to enrollment. Once it has been ascertained that subjects are eligible for the study, their authorization to use personal health information for this study and their informed consent will be obtained. Each subject will be assigned a unique study identification number at the time of study enrollment to ensure confidentiality of study data.

3.4.1 Inclusion Criteria

Subjects may be included in the study if they meet all of the following criteria:

1. Subjects must be 18 years or older.

2. Locally advanced or metastatic pancreatic ductal adenocarcinoma, with at least one target lesion in the abdomen measuring ≥ 1cm.

   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.

3.4.2 Exclusion Criteria

1. Patients who because of age, general medical or psychiatric condition, or physiologic status cannot give valid informed consent.

2. 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.

3. Poorly controlled hypertension, defined as either systolic >170 or diastolic >110. The addition of anti-hypertensives to control blood pressure is allowed for eligibility determination
4. Congestive Heart Failure ≥ Class III
5. Myocardial infarction within the past year
6. History of QT prolongation on EKG, defined as pretreatment QTs > 440 msec in males or > 460 msec in females
7. Pregnant and lactating females

3.5 Inclusion and Recruitment of Women and Minorities

Individuals of any sex/gender, race, or ethnicity may participate.

The study recruitment strategy aims to achieve representation of minority groups that reflects the demographics of the affected population in the catchment area.

3.6 Study Timeline

3.6.1 Primary Completion

The total planned accrual for this study is 40 patients. It is estimated that 1-2 subjects will be enrolled per month. It is estimated that 36 months will be required to complete accrual and data acquisition.

3.7 Study Completion

The expected study completion date is December 2023 after the study opens to accrual.

3.7.1 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.

The IRB must be promptly notified that the study will no longer be taking place and provided with a detailed written explanation.

4 Investigational Medicinal Product

4.1 Description, Supply and Storage of Investigational Medicinal Product

4.1.1 Hyperpolarized ^{13}C Pyruvate

^{13}C is a stable, non-radioactive isotope of carbon with approximately 1% natural abundance. [1-^{13}C]pyruvate has exactly the same chemical characteristics as pyruvate. In [1-^{13}C]pyruvate, the C-1 carbonyl 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 dynamic nuclear polarization (DNP) technique. As [1-^{13}C] pyruvate has the same chemical characteristics as pyruvate, it is metabolized the same way. The polarization procedure allows

MR imaging to rapidly detect the hyperpolarized ^{13}C-label in [1-^{13}C]pyruvate and its metabolites, [1-^{13}C]lactate, [1-^{13}C]alanine, and [1-^{13}C]bicarbonate.

Formulation

The formulation of IMP in this study will be compounded using 250 mM [1-^{13}C]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 SPINLabTM) (see Appendix 5), placed in an adjacent room to the MR suite, will then be used to generate the final hyperpolarized [1-^{13}C] 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 SPINLabTM instrument, and performing drug accountability. See preparation methods (below) and Appendix 5 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. Pre-filled sterile fluid paths will be stored at -20 degrees C, and thawed prior to clinical use.

Refer to Appendix 3 (Handling and Storage Safety Data Sheet) for information.

Preparation Methods

IMP will be compounded under the supervision of a trained pharmacist, using a mixture of [1-^{13}C] pyruvate and AH111501 sodium salt. The mixture will be used to pre-fill a sterile fluid path using laser-welding technique under aseptic conditions in a clean room adjacent to the MR scanner. The pre-filled sterile fluid path will be stored frozen at -20 degrees Celsius until the time of clinical use at the treatment unit in the Surbeck lab (1700 4th Street, Byers Hall, Rm 104-115, San Francisco, CA) 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 SPINLabTM instrument (see Appendix 5).

The hyperpolarization process will utilize the fully automated GE SPINLabTM hyperpolarizer, which will be situated in a room adjacent to the MR suite. The mixture of [1-^{13}C]pyruvate 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, diluted with TRIS/EDTA buffer solution, and forced through a C18

reverse-phase chromatographic column, which removes AH111501 to a level below 3 μM, all in an automated fashion using the pre-fabricated sterile fluid path and SPINLabTM 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 local 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 ~50 mL of 250 mM [1-^{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 (per Appendix 2) is then delivered via the infusion pump to the subject at a rate of 5 ml/second. Note that for different preparations of [1-^{13}C]pyruvate, the concentration may be slightly different than 250 mM. The current concentration and corresponding injection volumes are provided (Appendix 2). If a slightly different concentration of [1-^{13}C]pyruvate is available, the appropriate injection volume will be different but the amount of [1-^{13}C]pyruvate injected will be unchanged. If this course of action is taken, the table corresponding to 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.

For associated hazards and how to handle them, refer to Appendix 3 (Handling and Storage Safety Data Sheet).

Safety Information

Earlier studies of nonhyperpolarized pyruvate outside of the United States were summarized in section 1.2.1

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 findings from the phase I study of prostate cancer patients at UCSF, which included comprehensive safety monitoring, are 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 Treatment Plan

Dosage and Administration

Each subject will receive HP ^{13}C pyruvate injection at a dosage of 0.43 mL/kg body weight, the maximum administered dose in the phase I study. It will be injected intravenously at a rate of 5 mL/second followed by a 20 mL saline flush at 5 mL/second.

5.1 Early Stopping Rules for Safety

Toxicity will be reviewed on a continuous basis. In the event of one serious drug-related adverse event, enrollment will be halted for study safety review. 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.

6 Study Procedures and Observations

The study-specific procedures and assessments are detailed in this section and outlined in the Study Calendar – Section 6.1.

The Screening procedures and assessments must be completed within 28 days of IMP administration, unless otherwise noted. Any results falling outside of the reference ranges may be repeated at the discretion of the investigator.

All on-study visit procedures are allowed a window of ± 5 days unless otherwise noted. Visit delays for public holidays or weather conditions do not constitute a protocol violation.

6.1 Study Calendar

Period / Procedure	Screening	Baseline HP ^{13}C pyruvate MRI			Follow up HP ^{13}C pyruvate MRI[1] 4w ± 2w post tx initiation			Follow-Up
		Pre-scan	HP ^{13}C MR Imaging	Post-scan	Pre-scan	HP ^{13}C MR Imaging	Post-scan	2-3mo interval tx initiation
Study Day / Visit Day	**-28d - 0d**							
Study Tx / Drug Admin								
HP ^{13}C pyruvate injection			X			X[1]		
Administrative Procedures								
Informed consent	X							
Clinical Assessments								
Baseline conditions	X							
Concomitant medications	X							
Medical history/Demographics	X							
Performance status	X							
Vital signs	X	X		X[2]	X[1]		X[1,2]	
AE assessment				X[2]			X[1,2]	
Laboratory Assessment								
Serum creatinine	X							
Imaging Procedures								
MR Scan			X			X[1]		
Disease Assessment on SOC CT								X[1]

[1] Cohort B only: For patients in Cohort B, a follow up HP 13C pyruvate MRI will be obtained as per above procedures at 4 weeks (+/- 2 weeks) following the initiation of systemic therapy or radiotherapy as per clinical care.
[2] Vital signs will be collected at the end of the visit (once the final scan is completed). AEs will be assessed following each scan.

6.2 Participant Registration

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 participants consented to the study 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.

6.3 Schedule of Procedures and Assessments

6.3.1 Pretreatment Period

6.3.1.1 Screening Assessments

- Administrative Procedures
  - Informed consent
- Clinical Assessments
  - Vital signs including blood pressure and heart rate
  - Medical history and demographic information
  - Baseline conditions assessment
  - Concomitant medications
  - Documentation of disease assessment
  - Performance status
- Laboratory Assessments
  - Serum creatinine

6.3.2 Treatment Period

6.3.2.1 Study Procedures, Baseline HP ^{13}C pyruvate MRI

- Pre-Scan
  - Clinical Assessments
    - Vital signs including blood pressure and heart rate

      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 Scan

Patient is transferred to the MRI suite. Standard 1H MRI exam will be obtained in the abdomen, including T2 weighted imaging, and T1 weighted imaging pre and dynamically following gadolinium contrast administration (gadobutrol at 0.1ml/Kg body weight). If the patient has estimated glomerular filtration rate (eGFR) < 30, gadobutrol will not be administered.

- HP ^{13}C MR Imaging
    - Study Tx / Drug Admin: Hyperpolarized 13C pyruvate injection

      Hyperpolarized 13C pyruvate will be injected intravenously at a dose of 0.43 mL/kg and at a rate of 5 mL/second followed by a 20 mL saline flush at 5 mL/second. The volume to be injected is based upon body weight and is outlined in Appendix 2.
    - Imaging Procedures: Dynamic 13C MR imaging
        - 1-2 minutes post-injection
        - 13C 3D MRI sequence with Multiband spectral-spatial excitation pulse for minimal [1-13C] pyruvate saturation and echo-planar imaging (EPI) readout for accelerated spectral-spatial sampling.
        - An **optional second HP 13C pyruvate injection and MRI** acquisition will be performed within 15 to 60 minutes following completion of the first scan. This repeat injection and scan is optional. This second injection will only occur if there is no adverse reaction to the first injection. The optional second injection and MRI scan are used to assess the repeatability of tumor HP 13C pyruvate metabolism.
- Post-Scan
    - Clinical Assessments
        - Vital signs including blood pressure and heart rate at the end of the visit (when the last scan is complete).

          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.

          If the subject experiences an adverse event such as increased heart rate or decreased blood pressure that are deemed clinically significant by the monitoring MD or nurse on site, the subject will be monitored closely until all vital signs are stable.

          Therefore, the period for adverse event monitoring will be up to 24 hours. Notably, no significant adverse events have been observed in any of the other ongoing hyperpolarized 13C pyruvate MRI studies.
    - AE assessment: For all subjects, a MD or nurse will follow up with the subject with a phone call within 24 hours to assess for any side effects. AEs will also be assessed at the visit, after each scan is completed.

6.3.2.2 Study Procedures, Follow-Up HP ^{13}C pyruvate MRI (cohort B only)

For patients in Cohort B, a follow up HP 13C pyruvate MRI will be obtained as per above procedures at 4 weeks (+/- 2 weeks) following the initiation of systemic therapy or radiotherapy as per clinical care.

- Pre-Scan
    - Clinical Assessments
        - Vital signs including blood pressure and heart rate

          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 Scan

  Patient is transferred to the MRI suite. Standard 1H MRI exam will be obtained in the abdomen, including T2 weighted imaging, and T1 weighted imaging pre and dynamically following gadolinium contrast administration (gadobutrol at 0.1ml/Kg body weight). If the patient has estimated glomerular filtration rate (eGFR) < 30, gadobutrol will not be administered.
- HP ^{13}C MR Imaging
    - Study Tx / Drug Admin: Hyperpolarized 13C pyruvate injection

      Hyperpolarized 13C pyruvate will be injected intravenously at a dose of 0.43 mL/kg and at a rate of 5 mL/second followed by a 20 mL saline flush at 5 mL/second. The volume to be injected is based upon body weight and is outlined in Appendix 2.
    - Imaging Procedures: Dynamic 13C MR imaging
        - 1-2 minutes post-injection
        - 13C 3D MRI sequence with Multiband spectral-spatial excitation pulse for minimal [1-13C] pyruvate saturation and echo-planar imaging (EPI) readout for accelerated spectral-spatial sampling.
        - An **optional second HP 13C pyruvate injection and MRI** acquisition will be performed within 15 to 60 minutes following completion of the first scan. This repeat injection and scan is optional. This second injection will only occur if there is no adverse reaction to the first injection. The optional second injection and MRI scan are used to assess the repeatability of tumor HP 13C pyruvate metabolism.
- Post-Scan
    - Clinical Assessments
        - Vital signs including blood pressure and heart rate at the end of the visit (when the last scan is complete).

          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.

If the subject experiences an adverse event such as increased heart rate or decreased blood pressure that are deemed clinically significant by the monitoring MD or nurse on site, the subject will be monitored closely until all vital signs are stable.

Therefore, the period for adverse event monitoring will be up to 24 hours. Notably, no significant adverse events have been observed in any of the other ongoing hyperpolarized 13C pyruvate MRI studies.

- AE assessment: For all subjects, a MD or nurse will follow up with the subject with a phone call within 24 hours to assess for any side effects. AEs will also be assessed at the visit, after each scan is completed.

Reporting of MR Findings:

The HP 13C pyruvate MRI is considered a research test and as such, will not be used in the clinical management of patients enrolled on the study. If there are any clinically relevant abnormal findings detected on standard anatomic MR imaging, these results will be communicated to patient and treating health care provider within 24 hours of completion of MR scan.

6.4 Supportive Care

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 IMP 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. Any patient who becomes unstable will be stabilized and transferred to the hospital by ambulance.

7 Reporting and Documentation of Results

7.1 Evaluation of Efficacy (or Activity)

This is a pilot study to investigate whether Hyperpolarized ^{13}C-pyruvate MRI can provide metabolic information and tumor response assessment in patients with pancreatic ductal adenocarcinoma.

7.2 Evaluation of Safety

Analyses will be performed for all patients having received at least one dose of IMP. The study will use the CTCAE v5.0 for reporting of non-hematologic adverse events and modified criteria for hematologic adverse events.

7.3 Definitions of Adverse Events

7.3.1 Adverse Event

An adverse event (also known as an adverse experience) is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug 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 use of a drug, without any judgment about causality. An adverse event can arise from any use of the drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

Laboratory test value abnormalities will not be recorded as AEs unless they are designated as clinically significant, defined as any one of the following: symptomatic, requiring treatment, resulting in dose modification or delay or premature study withdrawal, or placing the subject at risk for other toxicity in the judgment of the treating investigator.

7.3.2 Adverse reaction

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

7.3.2.1 **Suspected**

A suspected adverse reaction is defined as any adverse event for which there is a reasonable possibility that the drug 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 drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

7.3.2.2 Unexpected

An adverse event or suspected adverse reaction is considered unexpected if it is not listed in the investigator brochure or package insert(s), or is not listed at the specificity or severity that has been observed, or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

“Unexpected,” as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

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 investigator brochure. For example, a certain number of non-acute deaths in a cancer trial would be anticipated as an outcome of the underlying disease, but such deaths would generally not be listed as a suspected adverse reaction in the investigator brochure.

Some adverse events are listed in the Investigator Brochure as occurring with the same class of drugs, or as anticipated from the pharmacological properties of the drug, even though they have not been observed with the drug under investigation. Such events would be considered unexpected until they have been observed with the drug under investigation. For example,

although angioedema is anticipated to occur in some patients exposed to drugs in the ACE inhibitor class and angioedema would be described in the investigator brochure as a class effect, the first case of angioedema observed with the drug under investigation should be considered unexpected for reporting purposes.

7.3.2.3 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.

7.3.2.4 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.

7.4 Recording of an Adverse Event

Refer to the Data Safety Monitoring Plan, located in Appendix 4.

7.5 Follow-up of Adverse Events

All participants who experience adverse events will be followed with appropriate medical management until resolved or stabilized, as determined by the investigator, or until the initiation of new anti-cancer therapy, whichever occurs first. 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 administration of the investigational drug was stopped, a re-challenge of the subject with the investigational drug may be conducted if considered both safe and ethical by the Investigator.

7.6 Adverse Events Monitoring

Refer to the Data Safety Monitoring Plan, located in Appendix 4.

7.7 Expedited Reporting

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 last administration of the IMP(s) and it is determined to be related either to the IMP(s) or to a 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 Committee on Human Research (Institutional Review Board)

The UCSF PI must report events to the UCSF IRB according to institutional guidelines.

UCSF IRB website for guidance in reporting adverse events: https://irb.ucsf.edu/adverse-event

Expedited Reporting to the FDA

If the study is being conducted under an IND, the Sponsor (or the Sponsor-Investigator) is responsible for determining whether or not the suspected adverse reaction meets the criteria for expedited reporting in accordance with federal regulations (21 CFR §312.32).

The Sponsor (or Sponsor-Investigator) must report in an IND safety report any suspected adverse reaction that is both serious and unexpected. The Sponsor needs to ensure that the event meets all three definitions:

- Suspected adverse reaction
- Unexpected
- Serious

If the adverse event does not meet all three of the definitions, it should not be submitted as an expedited IND safety report.

The timeline for submitting an IND safety report to FDA is no later than **15 calendar days** after the Investigator determines that the suspected adverse reaction qualifies for reporting (21 CFR 312.32(c)(1)).

Any unexpected fatal or life-threatening suspected adverse reaction will be reported to FDA no later than **7 calendar days** after the Investigator's initial receipt of the information (21 CFR 312.32(c)(2)).

Any relevant additional information that pertains to a previously submitted IND safety report will be submitted to FDA as a Follow-up IND Safety Report without delay, as soon as the information is available (21 CFR 312.32(d)(2)).

8 Statistical Considerations and Evaluation of Results

8.1 Sample Size Considerations

8.1.1 Sample Size and Power Estimate

For Cohort A: Assuming signal-to-noise ratio of target tumor ^{13}C pyruvate metabolism of 30 and standard deviation of 5, a sample size of 20 evaluable patients will provide an expected width of 95% confidence intervals of +/-2.2, with a standard error of 1.1.

For Cohort B: A sample size of 20 evaluable patients will allow the detection of a standardized difference of 0.38 with 80% power at a 2-sided significance level of 0.05 using a paired t-test.

8.1.2 Accrual Estimates

It is estimated that 1-2 subject will be enrolled per month, and approximately 36 months will be required to complete accrual and data acquisition to the study. In the case of subjects being unevaluable for analysis of primary endpoint, additional subjects may be enrolled to reach the required total number of evaluable subjects.

8.2 Imaging Analysis

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 multiparametric imaging data. Tumor regions of interest (ROIs) will be drawn to quantify MR biomarker (peak lactate/pyruvate ratio, lactate /pyruvate AUC, k_{PL}) values across the selected volumes of interest. The peak lactate/pyruvate ratio, lactate /pyruvate AUC, k_{PL} represent different methods of analyzing the pyruvate to lactate metabolic conversion.

8.3 Analysis Plans

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.

In this pilot study, we will focus on the primary PDA HP ^{13}C pyruvate metabolism (if present) because accurate assessment of treatment response in the primary PDA is particularly challenging on conventional imaging given its infiltrative nature(10). If the primary tumor has been resected, we will then focus our analysis on the largest abdominal metastasis that is amenable to HP MRI.

We will not explicitly adjust for multiple comparisons in analyzing the data in this pilot study.

8.3.1 Analysis Population

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

8.3.2 Primary Analysis

Cohort A: Signal-to-noise ratio of ^{13}C pyruvate metabolism measures (peak ^{13}C lactate/pyruvate ratio, ^{13}C lactate/pyruvate AUC ratio, and apparent rate constant for pyruvate-to-lactate conversion, k_{PL}) in the target tumor (primary tumor and/or abdominal metastases).

Cohort B: Percent changes in the target tumor (primary tumor and/or abdominal metastases) ^{13}C pyruvate metabolism measures (peak ^{13}C lactate/pyruvate ratio, ^{13}C lactate/pyruvate AUC ratio, and k_{PL}) between pre-treatment scan and scan obtained at 4-week (+/- 2 weeks) following treatment initiation.

Primary endpoint analysis: For Cohort A, the signal-to-noise ratio of the target lesion ^{13}C pyruvate metabolism measures will be determined for each patient. Descriptive statistics will be used to summarize the mean, standard deviation, and 95% confidence interval of the measurements.

For Cohort B, paired t-test or Wilcoxon signed rank test will be used to compare the target tumor HP ^{13}C pyruvate metabolism pre- and 4-week (+/- 2 weeks) post treatment initiation.

8.3.3 Secondary Analysis

Cohort A and B: Repeatability of ^{13}C pyruvate metabolism measures in the target tumor (primary tumor and/or abdominal metastasis) in patients with same-day repeated dose.

Cohort B: The association between the baseline or changes in the target tumor pyruvate metabolism at 4 weeks post treatment initiation and tumor best objective response as defined according to RECIST criteria on subsequent standard-of-care CT.

Secondary endpoint analysis: For Cohort A and B, intraclass correlation coefficient (ICC) will be used to estimate the intra-subject agreement to assess repeatability of tumor HP ^{13}C pyruvate metabolism in patients with same-day repeated dose.

ICC will be used to estimate agreement, and obtained from a one-way analysis of variance model based on 2 measurements per subject. The result will be presented with a 95% confidence interval. If 20 (out of 40) subjects had repeated dose of the hyperpolarized 13C pyruvate, we will have 90% power to show an ICC of at least 0.6.

For Cohort B, tumor best objective response will be defined using RECIST 1.1 on subsequent clinical CT scans. For the purpose of response assessment in this pilot study, we will group patients either as having disease control when the best response is complete response (CR), partial response (PR), or stable disease (SD) on subsequent clinical CT scans, or having disease progression when the best response is progressive disease (PD) on subsequent CT scans. Based on prior published studies (37, 38) in PDA patients who receive first- or second-line therapies (i.e. FOLFIRINOX, gemcitabine plus nab-paclitaxel, or gemcitabine monotherapy), we estimate an average 50% disease control rate for this group of patients. We will compare the baseline or changes in the target tumor ^{13}C pyruvate metabolism at 4 week (+/-2 weeks) after treatment initiation between the disease control group and disease progression group (as defined by RECIST on subsequent clinical CT scans) using the Mann-Whitney tests.

8.3.4 Exploratory Analysis

The correlation in HP ^{13}C pyruvate metabolism between the primary tumor and abdominal metastases (when present) at baseline and following treatment.

Exploratory endpoint analysis: Pearson correlation tests will be used to compare the HP ^{13}C pyruvate metabolism between the primary tumor and abdominal metastasis when present.

9 Study Management

9.1 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 study, 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.

The clinical investigation will not begin until either FDA has determined that the study under the Investigational Drug Application (IND) is allowed to proceed or the Investigator has received a letter from FDA stating that the study is exempt from IND requirements.

The Investigator must comply with the applicable regulations in Title 21 of the Code of Federal Regulations (21 CFR §50, §54, and §312), GCP/ICH guidelines, and all applicable regulatory requirements. The IRB must comply with the regulations in 21 CFR §56 and applicable regulatory requirements.

9.2 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 CHR (UCSF Institutional Review Board). Prior to obtaining CHR 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.

9.3 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.

9.4 Changes in the Protocol

Once the protocol has been approved by the UCSF CHR, 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 CHR 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 CHR approval. In this circumstance, however, the Investigator must then notify the CHR 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.

9.5 Handling and Documentation of Clinical Supplies

The Principal Investigator 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.

9.6 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. 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.

9.7 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. See Appendix 4, Data and Safety Monitoring Plan for additional information.

9.8 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, CHR 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.

10 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 Dosing Table

Injection Volumes (250 mM [1-13C] pyruvate) for Individual Body Weights (mL)

Body Weight (kg)	0.43 ml/kg	Body Weight (kg)	0.43 ml/kg	Body Weight (kg)	0.43 ml/kg
40	17	77	33	114	49
41	18	78	34	115	49
42	18	79	34	116	50
43	18	80	34	117	50
44	19	81	35	118	51
45	19	82	35	119	51
46	20	83	36	120	52
47	20	84	36	121	52
48	21	85	37	122	52
49	21	86	37	123	53
50	22	87	37	124	53
51	22	88	38	125	54
52	22	89	38	126	54
53	23	90	39	127	55
54	23	91	39	128	55
55	24	92	40	129	55
56	24	93	40	130	56
57	25	94	40	131	56
58	25	95	41	132	57
59	25	96	41	133	57
60	26	97	42	134	58
61	26	98	42	135	58
62	27	99	43	136	58
63	27	100	43	137	59
64	28	101	43	138	59
65	28	102	44	139	60
66	28	103	44	140	60
67	29	104	45	141	61
68	29	105	45	142	61
69	30	106	46	143	61
70	30	107	46	144	62
71	31	108	46	145	62
72	31	109	47	146	63
73	31	110	47	147	63
74	32	111	48	148	64
75	32	112	48	149	64
76	33	113	49	150	65

Appendix 3 Safety Data Sheets

Conforms to the EU Directive 91/155/, amended 93/112

Chemical product and company identification

Product name: Hyperpolarized 13C Pyruvate Solution for Injection.
Synonyms: Hyperpolarized Pyruvate (13C) Injection
Application: Diagnostic imaging Pyruvic acid (AH110896 or AH111710) is the active component, AH111501 is a processing aid. Tris/EDTA buffer solution is added during compounding to neutralize the pyruvic acid.
Supplier: GE Healthcare AS, Sigma Aldrich Corp.
Emergency number: UCSF: [redacted]

Composition and information on ingredients

Composition: Pyruvic acid (AH110896 and AH111710), C3H4O3 AH110896 is the 13-C enriched pyruvic acid; [1-13C]pyruvic acid. AH111710 is pyruvic acid (no enrichment). AH111501 sodium salt (EPA), Na3C64H87O18S12 Trometamol (C4H11NO3), Na2EDTA (C10H14N2Na2O8), NaOH
Contents: AH111501 is a stabilized radical with the following functional groups: carboxyl, methoxy, ethyl, benzyl, methyl and thiol. Pyruvic acid is an organic acid with the following functional groups: carboxyl, carbonyl and methyl. Tromethamol is the buffer component, Na2EDTA is added as a metal complexing agent, NaOH will provide a pH of 6-8 when mixed with the pyruvic acid.
Main ingredients: Tromethamol (C4H11NO3) CAS No. 77-86-1 Na2EDTA (C10H14N2Na2O8) CAS No. 6381-92-6 NaOH CAS No.1310-73-2 Pyruvic acid (C3H4O3) CAS No.127-17-3

Hazards identification

Emergency overview: The two drug kit components (Mixture of pyruvic acid and AH111501 sodium salt, and Tris/EDTA solution) are classified as corrosive due to low/high pH and may cause burns. For the final product, the AH111501 is chromatographically removed prior to injection into patients. Water for injection is used in the production process and will decrease all concentrations, and the final pH is in the range 6 - 8. The injection can be considered as harmless when used as intended. If administered intravenously in doses greater than 0.43 ml/kg body weight or at injection rates greater than 5 ml/s, the injection may cause increases in heart rate and blood pressure.
Potential adverse health effects: Not known
Skin: Avoid contact with skin
Eyes: Avoid contact with eyes
Inhalation: Avoid inhalation

Ingestion: Avoid ingestion
Hazard classification, Hazard symbols: Not known

First-aid measures

Skin contact: Remove contaminated clothing and shoes and flush with plenty of water
Inhalation: Move to fresh air
Eyes: Flush with water
Ingestion: Wash out the mouth with water

Fire-fighting measures

Flammability: Non-flammable
Fire Extinguishing Media: H2O, CO2 , or dry powder
Fire Fighting Procedures: Not known
Decomposition and Explosion Hazards: Not known

Accidental release measures

Personal protection: Wear protective clothing, chemical-resistant gloves, and eye protection.
Clean up procedures: Ventilate the area, wash with plenty of water after material pickup is complete.
Environmental precaution: Not known

Handling and storage

Handling precautions: Handle with care. Avoid contact with eyes and skin, inhalation and ingestion.
Storage conditions: The solution produced for injection is to be used within short time. For analytical purposes, storage cold or at room temperature (not frozen), protected against light in airtight container.

Exposure controls, personal protection

Ventilation: N.A.
Protective gloves: Use chemical-resistant gloves
Eye protection: Use safety glasses
Protective clothing: Use protective clothing (lab coat or similar).

Physical and chemical properties

Appearance, color: colorless to slightly colored, clear liquid	
Odor: No characteristic odor	
Specific gravity: Not known	
Melting point, C: Not known	Boiling point, C: Not known
Decomposition products: N.A.	
Solubility: soluble in water	
pH: 6-8	Concentration (weight- %): 2.2 % w/w pyruvate

Stability and reactivity

Stability: For analytical purposes, storage cold or at room temperature (not frozen), protected against light in airtight container.
Reactivity: Not known
Conditions or materials to avoid: Light and air

Appendix 4 Data and Safety Monitoring Plan for a Phase II Institutional Study

1. Oversight and Monitoring Plan

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

- Semiannual auditing (depending on study accrual)
- Review of serious adverse events
- Minimum of biennial regulatory auditing

2. Monitoring and Reporting Guidelines

Investigators will conduct a continuous review of data and participant safety at monthly site committee meetings where the results of each participant's treatment are discussed and documented in the site committee minutes.

All institutional Phase II and III studies are designated with a moderate risk assessment. The data is audited semiannually with a random selection of twenty percent of the participants audited (or at least three participants if the calculated value is less than three). The DSMC Monitor/Auditor will audit a maximum of 5 cycles of treatment in the participants selected for the review, or until the selected participants discontinue study participation or the trial is closed with the IRB. Additionally, the assigned DSMC Monitor/Auditor will review no more than a total of 10 participant charts during the course of auditing this trial. DSMC Monitor/Auditors will send a follow-up report to the study team within 20 business days after the auditing visit is complete for the PI and the study team to resolve all action items from this report within 20 business days. Additionally, a regulatory audit will occur on a biennial basis to review all regulatory documents for the trial.

3. Review and Oversight Requirements

3.1 Adverse Event Monitoring

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

Adverse events are graded according to the Common Terminology Criteria for Adverse Events (CTCAE) as developed and revised by the Common Therapy Evaluation Program (CTEP) of the National Cancer Institute. Adverse events are further given an assignment of attribution or relationship to investigational agent or study procedure. Attribution categories are:

- Definite – The adverse event is clearly related to the investigational agent(s) or study procedure.
- Probable – The adverse event is likely related to the investigational agent(s) or study procedure.
- Possible – The adverse event may be related to the investigational agent(s) or study procedure.
- Unrelated – the adverse event is clearly not related to the investigational agent(s) or study procedure.

All Grade 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 attribution assignment.

3.2 Serious Adverse Event Reporting

By definition, an adverse event is defined as a serious adverse event (SAE) according to the following criteria:

- Death.
- Life-threatening (i.e. results in an immediate risk of death).
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Permanent or significant disability/incapacity
- Gives rise to a congenital anomaly/birth defect, or cancer, or any experience that suggests a significant hazard, contraindication, side effect, or precaution that may require medical or surgical intervention to prevent one of the outcomes listed above.
- Event occurring in a gene therapy study.
- Event that changes the risk/benefit ratio of a study.
- Any other event the Principal Investigator judges to be serious or which would suggest a significant hazard, contraindication, side effect, or precaution.

Serious adverse event reporting will be in accordance with all IRB regulations. For trials conducted under an investigational new drug (IND) application, the SAE will be reported in accordance with Code of Federal Regulation Title 21 Part 312.32 and will be reported on a Med Watch form.

UCSF IRB website for guidance in reporting serious adverse events:

https://irb.ucsf.edu/adverse-event

Med Watch forms and information:

www.fda.gov/medwatch/getforms.htm

All serious adverse events are entered into OnCore®, as well as submitted to the IRB (per IRB guidelines). The SAEs are 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. The date the SAE is sent to all required reporting agencies will be documented in OnCore®.

If the SAE involves a subject death, and is determined to be possibly, probably or definitely related to the investigational drug or any research related procedure, the event must be reported to the DSMC Chair (or Vice Chair) and DSMC Director within one business day.

3.3 Review of 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, the Principal Investigator will notify the DSMC via report 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.

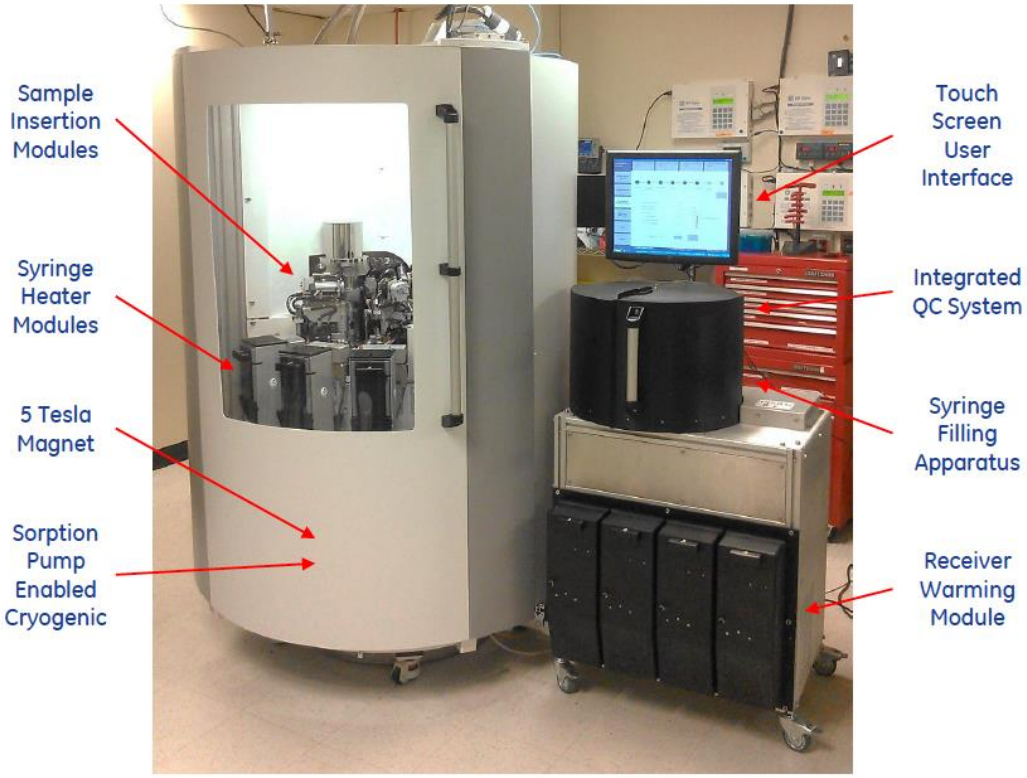
If at any time the Investigator voluntarily holds enrollment or stops the study due to safety issues, the DSMC Chair (or Vice Chair) and the DSMC Director must be notified within one business day and the IRB must be notified as per IRB reporting regulations.

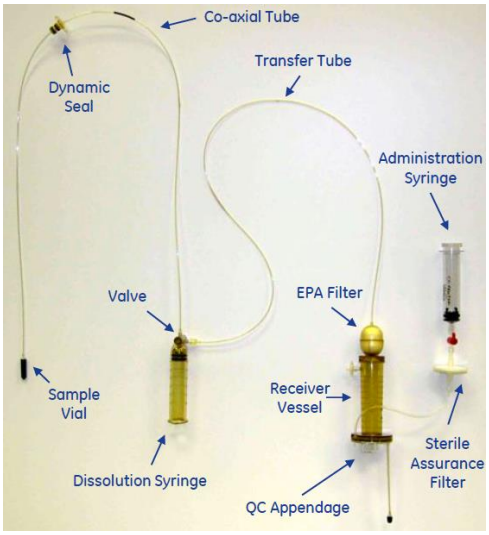
Data and Safety Monitoring Committee Contacts:

[redacted] (DSMC Chair)
[redacted]
[redacted]
[redacted]
UCSF HDFCCC
San Francisco, CA 94158

[redacted] (DSMC Director)
[redacted]
[redacted]
[redacted]
UCSF HDFCCC
San Francisco, CA 94143

Appendix 5 GE SPINLab™ Hyperpolarizer and Sterile Fluid Path





TOP: SPINLab™ hyperpolarizer will produce hyperpolarized [1-^{13}C] pyruvate in an automated fashion with an integrated QC system for validation of IMP prior to each patient injection.
RIGHT: Sterile fluid path. The sample vial will be filled with [1-^{13}C] pyruvic acid and AH111501 and laser welded to the pre-fabricated sterile fluid path under aseptic conditions. The pre-filled sterile fluid path will then be loaded into the SPINLab system for automated preparation of hyperpolarized [1-^{13}C] pyruvate, which is collected in an administration syringe for rapid patient injection.