Title: Evaluating Hyperpolarized and Proton Brain Metabolism in Patients with Glioblastoma

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Protocol Signature Page

1. I agree to follow this protocol version as approved by the UCSF Protocol Review and Monitoring Committee (PRMC), Institutional Review Board (IRB), and Data and Safety Monitoring Committee (DSMC).
2. I will conduct the study in accordance with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practices (GCP) and the applicable IRB, ethical, federal, state, and local regulatory requirements.
3. I certify that I, and the study staff, have received the required 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.

[redacted]

Signature of UCSF Principal Investigator

08/22/2023

Date

Susan Chang, MD

Name of UCSF Principal Investigator (Printed)

1 SYNOPSIS

Name of product manufacturer:
GE Healthcare Ltd. and Sigma Aldrich Corp.

Name of Finished Product:
Hyperpolarized Pyruvate (^{13}C) Injection

Name of Active Ingredient:
[1-^{13}C] pyruvic acid (AH-110896)

Phase of Development: Phase 1

Number of Subjects: 90 subjects

Objectives:

Primary:

1) To evaluate metabolic changes in the residual tumors in response to standard therapy in thirty patients with newly diagnosed glioblastoma (GBM). The hyperpolarized ^{13}C data and ^{1}H magnetic resonance spectroscopic imaging (MRSI) will be obtained after initial surgery and before receiving standard of care treatment with radiation therapy (RT) and temozolomide (TMZ) and at the first post-RT follow-up scan.

2) To examine metabolic markers of recurrent tumor and treatment related effects in patients with recurrent GBM. The hyperpolarized ^{13}C data and ^{1}H MRSI will be obtained from thirty patients with suspected recurrent GBM prior to surgery and used to define targets for tissue sampling. The histological and molecular characteristics of the samples will be evaluated and compared with corresponding metabolic parameters.

3) To assess the clinical value of using hyperpolarized ^{13}C metabolic imaging for detecting early response to standard therapy in patients with recurrent GBM. Thirty patients with suspected recurrent glioblastoma who are being treated with standard of care therapies will be studied prior to treatment (^{13}C + ^{1}H), at 7 days (^{13}C + ^{1}H) and 6-8 weeks after initial therapy (^{1}H only).

Study Design:

The objective of this study is to combine hyperpolarized ^{13}C imaging and ^{1}H MRSI data to detect differences in dynamic and steady state metabolism that distinguish regions of normal brain, tumor and treatment related effects, and that can be applied to improve the clinical evaluation of patients with GBM. This hyperpolarized pyruvate (^{13}C) study includes the acquisition of magnetic resonance (MR) data and will be performed in patients with GBM. A total of ninety patients will be evaluated to establish the clinical relevance of obtaining ^{13}C hyperpolarized and ^{1}H MR metabolic measurements in patients with GBM. Thirty newly diagnosed patients (Cohort 1) will be studied both at baseline and after the completion of standard RT, thirty recurrent patients (Cohort 2) will be studied prior to surgical resection, and the other thirty recurrent patients (Cohort 3) will be studied at the baseline, 7 days and 6-8 weeks after initial therapy.

Selection of Subjects:

Inclusion Criteria

For Patients in Cohort 1: Histologically proven newly diagnosed GBM who will undergo standard of treatment with RT and TMZ.

For Patients in Cohort 2: Histologically proven recurrent suspected GBM who will receive surgical resection for the recurrence.

For Patients in Cohort 3: Histologically proven recurrent suspected GBM who will undergo standard treatment for the recurrence.

To be included in the study all subjects must also meet the following criteria:

(1) Patients must be > 18 years old and with a life expectancy > 16 weeks.
(2) Patients must have a Karnofsky performance status of ≥ 70.
(3) Patients must have adequate renal function (creatinine < 1.5 mg/dL) before imaging. These tests must be performed within 60 days prior to Hyperpolarized Imaging scan.
(4) Patients must not have any significant medical illnesses that in the investigator's opinion cannot be adequately controlled with appropriate therapy, would compromise the patient's ability to participate in this study or any disease that will obscure toxicity or dangerously impact response to the imaging agent.
(5) Patients must not have New York Heart Association (NYHA) Grade II or greater congestive heart failure
(6) Patients must not have history of myocardial infarction or unstable angina within 12 months prior to study enrollment.
(7) This study was designed to include women and minorities but was not designed to measure differences of intervention effects. Males and females will be recruited with no preference to gender. Minorities will actively be recruited to participate. No exclusion to this study will be based on race.
(8) Patients must sign an informed consent indicating that they are aware of the investigational nature of this study. Patients must sign an authorization for the release of their protected health information.
(9) Patients may not be known to be HIV-positive. HIV testing is not required for study participation.
(10) Patients must not have a history of any other cancer (except non-melanoma skin cancer or carcinoma in-situ of the cervix), unless in complete remission and off of all therapy for that disease for a minimum of 3 years.
(11) Patients must not be pregnant or breast feeding. Women of childbearing potential are required to obtain a negative pregnancy test within 14 days of Hyperpolarized Imaging scan. Effective contraception (men and women) must be used in subjects of child-bearing potential.

Exclusion Criteria

Subjects must be excluded from participating in this study if are not able to comply with study and/or follow-up procedures.

Investigational Medicinal Product: Hyperpolarized pyruvate (^{13}C) injection at a dosage of 0.43 mL/kg body weight, the maximum administered dose in the phase I study, will be administered. It will be injected intravenously at a rate of approximately 5 mL/second followed by a 20 mL saline flush at the same rate.

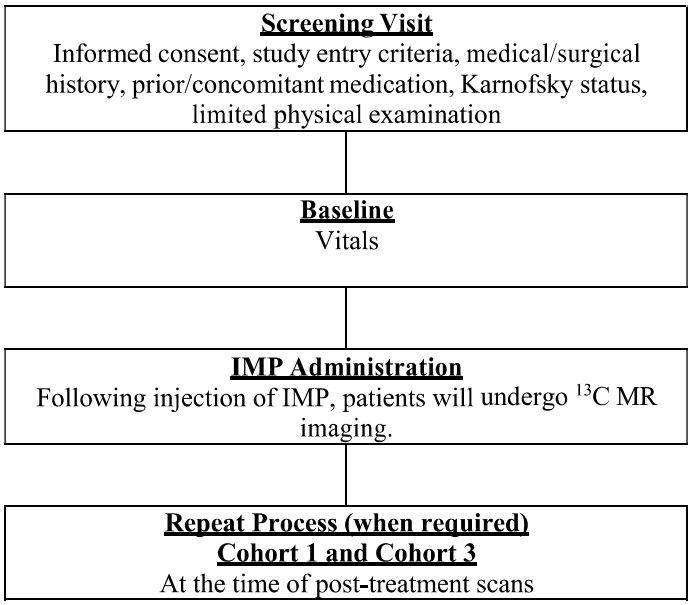
Duration of Study: For cohort 1 and 3, each subject will participate in the study for a maximum of 4 months. This consists of a screening period, baseline scan, where appropriate a repeat scan up to 4 weeks following completion of radiation (cohort 1) or initial treatment (cohort 3). For cohort 2, the duration of study will be a maximum of 7 days following the completion of the scan, depending on the schedule of surgery.

Imaging Safety, Feasibility, Reproducibility and Therapy Response Endpoints

1) Safety and feasibility of obtaining serial hyperpolarized ^{13}C MR metabolic imaging data from the brain of patients with recurrent glioblastoma.
2) The changes in ^{13}C parameters from baseline to the post-RT scan will be compared in the normal appearing brain, enhancing lesion, non-enhancing lesion and the choline-to-NAA index (CNI) lesion.
3) The ^{13}C metabolic parameters from the recurrent lesions will be compared to those in the regions of treatment related effects.
4) The ^{13}C and ^{1}H metabolic parameters will be evaluated to determine the association to time to progression and overall survival.

Study Plan:
All subjects who are enrolled in the study will receive hyperpolarized pyruvate (^{13}C) injection. The safety data will be analyzed using descriptive statistics for the entire study cohort tabulation of summary statistics, graphical displays, and any statistical analysis will be performed using statistical software such as Stata® or Statistica®. The last pre-administration observation will be used as the baseline value for calculating post-administration changes from baseline. All individual subject data (no patient identifiers) will be presented in separate data listings.

Study Diagram



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3 LIST OF ABBREVIATIONS

ACLS	Advanced Cardiac Life Support
AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body mass index
BW	Body Weight
CEL	Contrast enhancing lesion
CNI	Choline-to-NAA-index
CRC	Clinical research coordinator
CRF	Case report form
CTCAE	Common Terminology (Toxicity) Criteria for Adverse Events
CTMS	Clinical trials management system
DLT	Dose-limiting toxicity
DNP	Dynamic nuclear polarization
DSMC	Data Safety Monitoring Committee
EKG	Electrocardiogram
EPA	Electron paramagnetic agent
GBM	Glioblastoma multiforme
GCP	Good Clinical Practice
GE	General Electric
HDFCCC	Helen Diller Family Comprehensive Cancer Center
HIV	Human immunodeficiency virus
IMP	Investigational medicinal product
IRB	Institutional Review Board
MR	Magnetic resonance
MRI	Magnetic resonance imaging
MRSI	Magnetic resonance spectroscopic imaging
MTD	Maximum tolerated dose
NCI	National Cancer Institute
NEL	Non enhancing lesion
NOAEL	No-observed-adverse-effect level
NYHA	New York Heart Association
PRC	Protocol Review Committee
ROI	Region of interest
SAE	Serious adverse event
SNR	Signal to noise ratio
SOP	Standard operating procedure
T2L	T2 hyperintensity
TMZ	Temozolomide
UCSF	University of California at San Francisco
XRT	External radiation therapy

4 INTRODUCTION

Gliomas make up 80% of malignant brain tumor in adults[1,2] and are highly hetereogenous in terms of neuro-imaging and clinical outcome[3-7]. The primary treatment is surgical resection but these lesions are infiltrative in nature and recurrence typically occurs close to the site of the original tumor[4]. For patients with a diagnosis of glioma based upon WHO II criteria[3], the standard of care after resection is radiation and temozolomide (TMZ)[8,9]. Serial MR imaging is used to direct surgical resection, assess residual disease and monitor disease progression[10,11]. Predicting the efficacy of therapy and developing reliable response criteria is challenging using conventional imaging methods[12-30].

5 BACKGROUND

5.1 Rationale for Use of [1-^{13}C] Pyruvate

The study that is being proposed will utilize the delivery and metabolism of pyruvate within the brain. Animal models have shown that tumors have an increased rate of glucose and pyruvate metabolism relative to normal tissue. The increased glycolysis provides the cancer cells with an energy source that is independent of its oxygen supply, a carbon source for the biosynthesis of cell membranes that begins with lipogenesis, and an acid source that likely enables the cells to invade neighboring tissue. This results in increased levels of lactate. [1-^{13}C]pyruvate is a stable, non-radioactive isotopomer of pyruvate, which differs only in that the C-1 carbonyl has been replaced by a ^{13}C-nucleus that has a magnetic moment, can be hyperpolarized in the presence of an electron paramagnetic agent (EPA) by Dynamic Nuclear Polarization (DNP)[31] and visualized sensitively using carbon-13 (^{13}C) magnetic resonance spectroscopy imaging (MRSI). Hyperpolarized ^{13}C MR metabolic imaging is a promising new technology that provides an increase in sensitivity over standard approaches of more than 10,000-fold and monitors changes in functional properties of the tumor rather than anatomic features[31-36]. The conversion of pyruvate to alanine, bicarbonate, and lactate can be measured in the brain and surrounding tissues within seconds using MR methods.

5.2 Pre-clinical experience

A pre-clinical program has been performed in which the safety of both [1-^{13}C]-pyruvate and the EPA AH111501 (the 2 novel components in pyruvate (^{13}C) injection) has been demonstrated. The program included expanded acute-dose studies in Sprague-Dawley rats from which the No-Observed-Adverse-Effect-Levels (NOAEL) were estimated as 1784 mg/kg body weight (bw) for pyruvate and 34 mg/kg bw EPA for the 500 mM formulation of pyruvate and 892 mg/kg bw for pyruvate and 17 mg/kg bw for EPA for the 250 mM formulation (the lower NOAELs merely reflect the fact that lower doses of the 250 mM formulation were given as maximum dosing volumes were limited to 20 ml/kg bw for rats for both formulations) (Investigators' Brochure). The 500 mM pyruvate formulation was also tested in an expanded acute-dose study in beagle dogs; the NOAEL for pyruvate was 446 mg/kg bw and 8.5 mg/kg bw for EPA. In addition, the effects of pyruvate and EPA have been studied in local tolerance studies, genotoxicity studies, and central nervous system studies. The cardiovascular effects of formulations of pyruvate were studied in conscious and pentobarbital/fentanyl-anaesthetized dogs. The conscious dog was considered the most

relevant model for extrapolating results to healthy human volunteers and the anaesthetized dog as a sensitive model for extrapolating results to subjects with a compromised baroreflex function as may be experienced in elderly patients.

Based on the cardiovascular effects (vasodilatation leading to small, short-lasting, but reproducible reductions in blood pressure and compensatory increases in heart rate) observed in the most sensitive model, the NOAEL for Pyruvate Injection is 1.4 ml/kg bw (or 100 ml for a 70 kg subject) of a 250 mM formulation (equal to 31 mg/kg bw of pyruvate). A 250 mM formulation will be used in the study described in this protocol.

5.3 Preclinical brain tumor experience

The presence of high levels of lactate in subjects with brain tumor has been observed using in vivo MR spectroscopy techniques[37,38]. This was the basis for applying [1-^{13}C]pyruvate imaging to a orthotopic rodent model of GBM. Studies in control, U87 and U251 xenograft rat models of glioma have confirmed that hyperpolarized ^{13}C labeled pyruvate crosses the blood brain barrier and demonstrates significant differences in lactate, pyruvate and total carbon, as well as the ratio of lactate/pyruvate[39-40]. Figure 1 shows data obtained from two U-87 rats using a compressed sensing pulse sequence[41] and reconstruction that gives spectra with a 2x2x5mm spatial resolution in 10 seconds. The high resolution provides multiple voxels within the lesion and shows elevated lactate/pyruvate in enhancing tumor, as well as relatively low signal in the region of necrosis. This heterogeneity was confirmed by histological analysis of the tumor after removal.

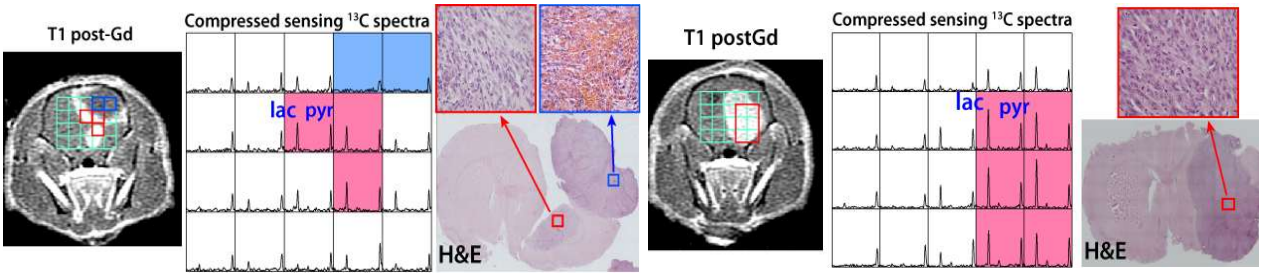


Figure 1. ^{13}C spectra and histological correlates in U87 rat xenographs after injection of hyperpolarized pyruvate using a compressed sensing data acquisition.

The effect of TMZ on the conversion of pyruvate to lactate was assessed in U87 tumors[42]. MR studies were performed as described previously[39]. ^{13}C MRSI and magnetic resonance imaging (MRI) data were acquired prior to treatment and then daily for one week. The ^{13}C data were acquired using a double spin-echo sequence at the time point when hyperpolarized ^{13}C lactate was found to be at a maximum with a spatial resolution of 0.086 cm^3 and acquisition time of 17s. Lactate/pyruvate increased in vehicle-treated control U87 tumors (Figure 2a), but dropped rapidly and significantly following TMZ treatment (Figure 2b). The increased on day 1 was by an average 20± 25 % in control rats but dropped 21± 22 % in TMZ-treated rats, resulting in a significant difference between the control and treated groups ($p<0.008$) Importantly, shrinkage of the anatomic lesion in TMZ-treated tumors was not seen until day 6. In contrast, data from tumors that were formed by U87 cells engineered to overexpress MGMT (U87-MG) and be TMZ resistant (Figure 2c) indicated that neither lactate/pyruvate nor tumor growth were affected by TMZ. These preliminary data indicate that a drop in lactate/pyruvate can serve as an early biomarker of response to TMZ and highlight the

potential for impacting patient care (37-39). These results provide strong evidence for the concept of using pyruvate (^{13}C) injection in patients with brain tumor to assess abnormal tumor metabolism and monitor tumor response to therapy.

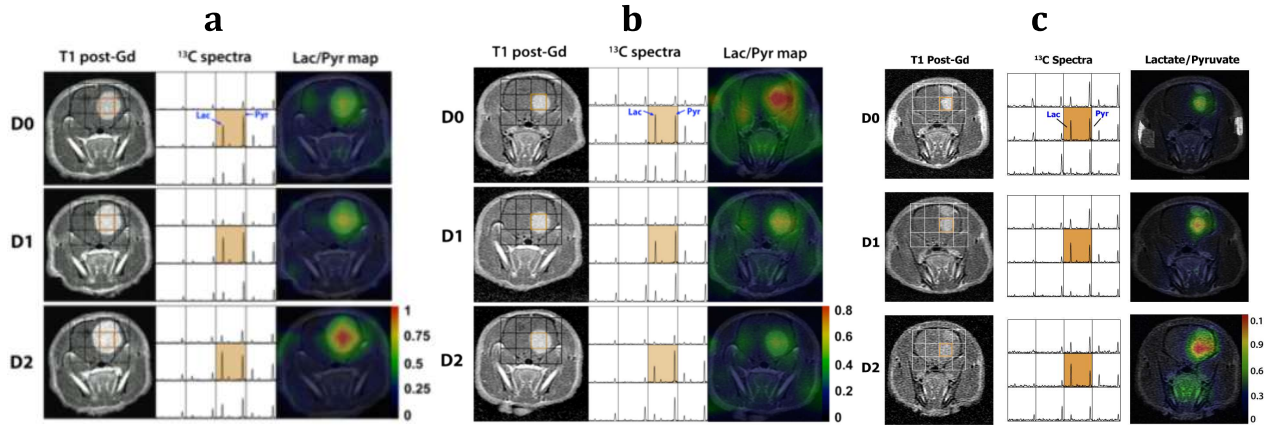


Figure 2. The change in lactate/pyruvate at day 1 (D1) and day 2 (D2) compared to pretreatment (D0) in vehicle-treated control U87 (a), TMZ-treated U87 (b), and U87-MGMT (c) tumors. Lactate/pyruvate drops immediately after treatement in drug sensitive, MGMT deficient U87 tumor (b), but increaseses with time in drug resistant, MGMT proficient tumors (c) as in untreated U87 (a).

Non-human primate brain imaging studies were performed at University of California at San Francisco (UCSF). The objective of these was to perform experiments that would evaluate the feasibility of observing metabolic changes using the dose of a 250 mM formulation of hyperpolarized pyruvate (^{13}C) injection in a 9-year old female cynomolgus monkey. These studies were designed to provide a realistic test of the methodology that will be applied in exploratory imaging studies in humans brains, as the same coil geometry and data acquisition parameters were used for the non-human primate brain as are being planned for human brains. Specific goals of the study were, therefore, to test the improved radiofrequency coils and pulse sequences, to determine the dynamics of arrival of the [1-^{13}C]pyruvate bolus, to evaluate the signal to noise ratio (SNR) of [1-^{13}C]pyruvate in healthy non-human primate brain, and to investigate whether its metabolic products could be observed at the NOAEL for pyruvate (1.4 ml/kg).

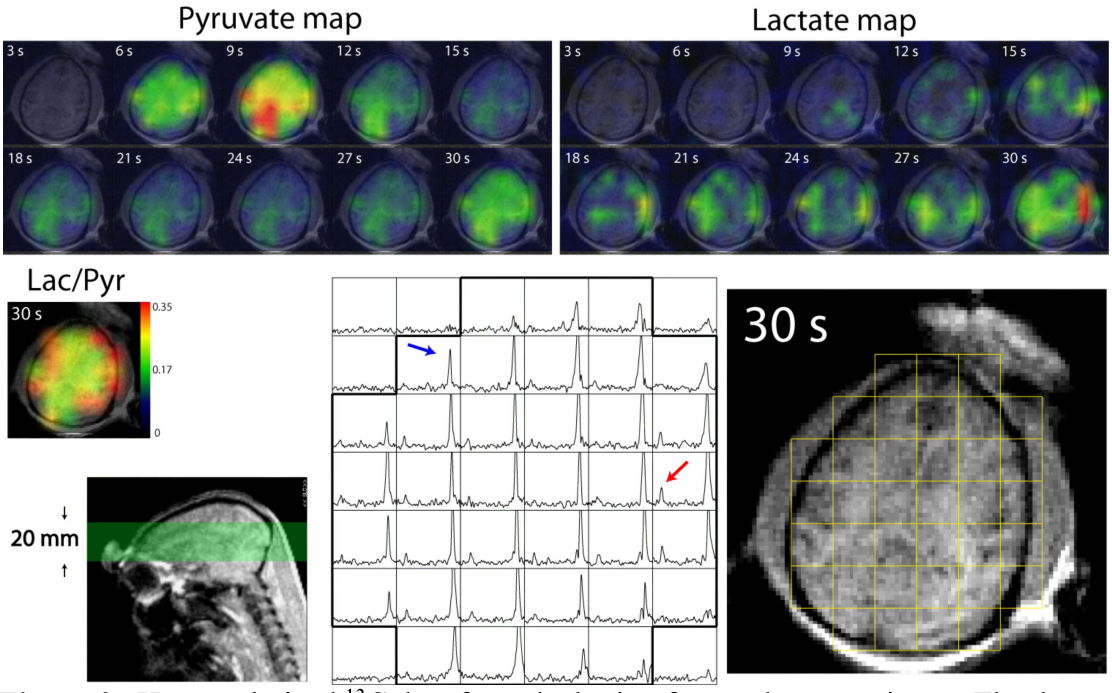


Figure 3. Hyperpolarized ^{13}C data from the brain of a non-human primate. The dynamic 2D spectral arrays were obtained with a variable flip angle. The red arrow indicates the lactate peak and the blue arrow the pyruvate peak.

The non-human primate studies demonstrated that high spatial and temporal resolution hyperpolarized [1-^{13}C] pyruvate images could be acquired from the brain using the coils and fast ^{13}C MRSI sequences designed for human brain studies (Figure 3 and Figure 4). The dynamic 2D localized spectral data were acquired with a time resolution of 3s. The overlaid images on the left panel are the time course of pyruvate and those on the right panel are the time course of lactate. The single color overlay image is the ratio of lactate/pyruvate at 30s post injection. The data come from a 20mm thick slab through the brain and were acquired with an in-plane resolution of 1cm using the 8 channel receive array. The maximum SNR of pyruvate was 173:1 and of lactate was 28:1. The pyruvate signal in the brain reached its maximum at 9s from the end of injection, while the maximum lactate signal (corrected for the variable flip angle) appeared at 18s (Figure 4). Figure 4 shows the overlay image of apparent rate constants for the conversion of pyruvate to lactate and the examples of time courses of pyruvate and lactate with the corresponding curve fits that were corrected for the variable flip angles. Signals from pyruvate and lactate were observed in both the brain and the surrounding tissues. The excellent SNR of pyruvate and lactate within normal brain indicates that pyruvate is able to cross the blood brain barrier and provide signals that can be measured using the pulse sequences and coils that we have developed.

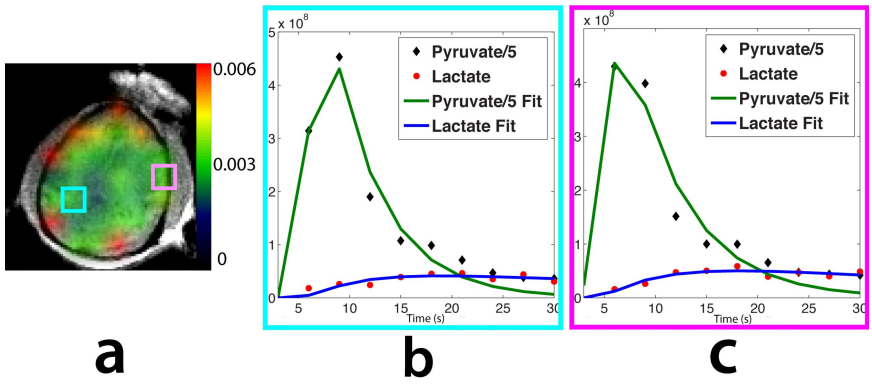


Figure 4. The quantified kinetics from the brain of a non-human primate: (a) The map of apparent rate constants for the conversion of pyruvate to lactate from a non-human primate brain. (b,c) Time courses of pyruvate and lactate, which were corrected for the variable flip angle scheme, and the corresponding curve fits within (b) and outside (c) brain.

5.4. Conclusions from pre-clinical experience

Based on the overall results from the non-human primate studies, the pyruvate injection dose of 1.4 ml/kg bw was well tolerated and no adverse effects were observed. However, data from the pre-clinical studies in conscious and anaesthetized dogs indicated that attention should be paid in initial human studies to the possibility of mild cardiovascular effects occurring soon after injection, similar to those that were reported when large volumes of pyruvate injection were administered rapidly to dogs (i.e., vasodilatation leading to small, short-lasting but reproducible reductions in blood pressure and compensatory increases in heart rate). The preclinical brain tumor studies demonstrated the ability of this imaging technique to monitor and detect changes in pyruvate metabolism in response to therapy.

5.5 Experience in humans

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 (Investigators' Brochure). In the original protocol it was planned that doses up to 0.71 ml/kg 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 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 unblinding 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" 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 <u>NOT</u> 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-SAEs that occurred among 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 30 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" 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.

5.6 UCSF Phase I experience in prostate cancer patients

Safety Results:

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. In this study, three dose levels of pyruvate injection, 0.14 ml/kg bw, 0.28 mg/kg bw, and 0.43 ml/kg bw were evaluated. Thirty-one patients underwent successful injection and imaging. Their characteristics are summarized in the table below.

Patient characteristics: phase I study

N	31
Median age (range)	63 (45-75)
Median PSA	5.9 (1.88-20.2)
Median LDH	141 (109-261)

Gleason score:	N
6	23
7	6
8	2

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	Subjects	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]
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 dosage for phase 2of the study. 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.

Imaging results:

Data from the phase I study in prostate cancer patients supported the concept of using pyruvate (^{13}C) injection in patients with cancer to assess abnormal tumor metabolism and possibly monitor tumor response to therapy. 31 patients received an injection of 250 mM hyperpolarized [1-^{13}C] pyruvate, followed by ^{13}C dynamic and MRSI at 3T. All patients were involved in an active surveillance protocol and had a multi-parametric ^{1}H MR exam[43] prior to their hyperpolarized ^{13}C MR study. One and two-dimensionally localized dynamic hyperpolarized data showed uptake of hyperpolarized pyruvate approximately 20 seconds after injection and demonstrated that the T_1 was adequate to detect uptake and conversion to lactate for 60 seconds which corresponded well with prior transgenic adenocarcinoma of the mouse prostate (TRAMP)[44] and human TSC data (Figure 5). No dose limiting toxicities were observed. The highest dose of hyperpolarized pyruvate (0.43mL/Kg) yielded the best pyruvate and lactate SNR in regions of cancer (mean, range 116, 42 to 290, and 20.4, 11 to 46, respectively) and the best contrast for tumor relative to normal prostate. Fast 2 and 3D based ^{13}C MRSI data demonstrated the ability to map the presence and spatial extent of prostate cancer based on elevated hyperpolarized lactate (Figure. 6).

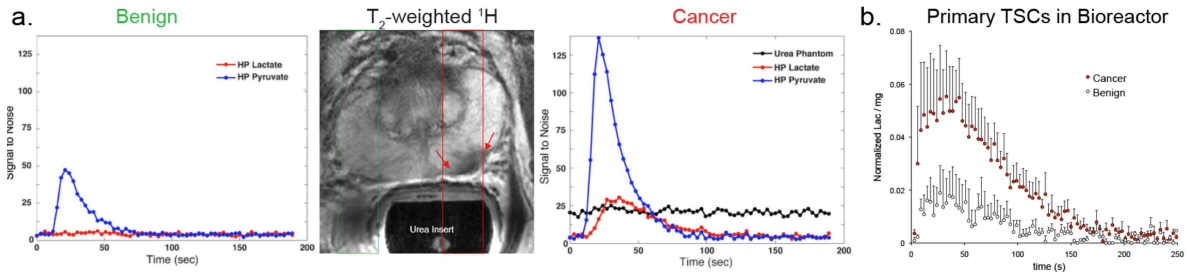


Figure 5. Hyperpolarized dynamics: (a) Representative hyperpolarized dynamics from benign and cancer regions of a patient. (b) Analogous TSCs in a bioreactor. We will utilize ^{13}C 2D MRSI sequence with a Multiband spectral-spatial excitation pulse for minimal [1-^{13}C]pyruvate saturation and echo-planar spectroscopic imaging (EPSI) readout for accelerated spectral-spatial sampling.

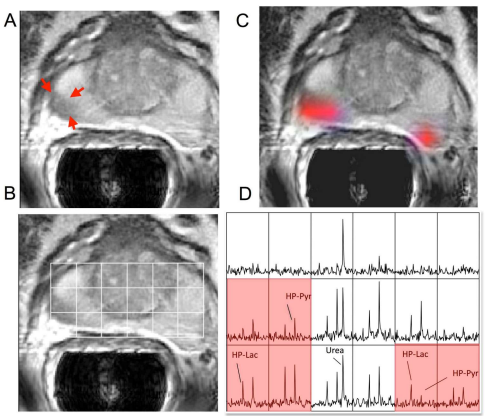


Figure 6. T_2 MR and hyperpolarized lactate images: T_2 MR image (A) with overlaid spectral grid (B) and corresponding ^{13}C MRSI array (D) and overlaid hyperpolarized lactate image (C) from a patient with a PSA of 4.58 ng/ml, biopsy proven cancer (Gleason 3+3, 8/12 cores) bilaterally. The biopsy proven cancer in the left lobe was missed on multiparametric MRI but identified on hyperperpolarized ^{13}C MR.

5.7 Preliminary experience at UCSF in patients with glioma

Safety Results:

The first study in patients with glioma is currently underway at UCSF and to date a total of 40 ^{13}C scans have been performed. There have been no adverse events for these subjects.

Imaging Results:

We have successfully developed and implemented experimental strategies for using [1-^{13}C] pyruvate to probe in vivo metabolism for patients with glioma[49]. Figure 7 gives an example of interpolated hyperpolarized ^{13}C metabolite ratio image from patients with glioblastoma. We have been developing and evaluating several MR pulse sequences and parameters. Options that have been considered include slice select, dynamic non-localized ^{13}C MRS, 2-D and 3-D dynamic ^{13}C MRSI, and multi-slice dynamic frequency specific ^{13}C echo planar imaging. A variety of different spatial resolutions and flip angle schemes have been applied in order to examine how they impact the observed signal to noise and metabolite ratios. These results are promising in terms of being able to use similar methods to evaluate changes in metabolic processes associated with tumor progression and treatment effects.

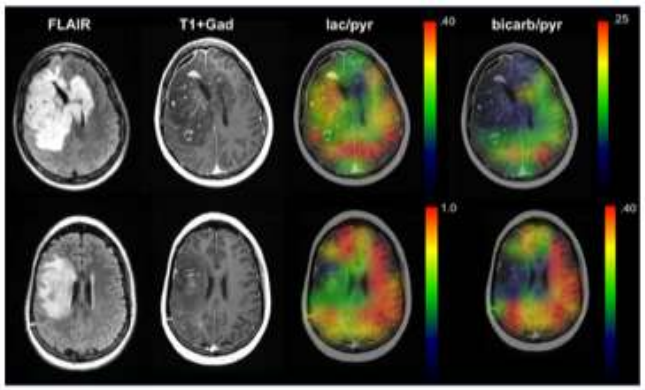


Figure 7. Anatomical and interpolated hyperpolarized ^{13}C metabolite ratio image overlays estimated from 2D EPSI dynamic data acquired at 2cm in plane resolution and 2-cm slice thickness from 2 patients with treated glioblastoma.

6 SUMMARY AND STUDY RATIONALE

Results from our group obtained in pre-clinical studies of U87 xenograft brain tumors demonstrated that ^{13}C labeled pyruvate can cross the blood brain barrier and can be used to monitor the rate and magnitude of conversion to lactate[39,40,42,45], which is elevated in tumors due to increased activity of the enzyme lactate dehydrogenase (LDH). Of particular interest is our observation that reductions in the ratio of lactate/pyruvate can be observed in as little as 1-2 days after treatment with TMZ[42]. Pre-clinical studies from other groups have observed significant reductions in lactate/pyruvate for treatment with radiation[46]. As the combination of these two treatments has been found to be more effective in patients with glioma than each alone, it can be expected to have a major effect on lactate/pyruvate. While the ability of hyperpolarized [1-^{13}C] pyruvate to reflect metabolic changes in response to radiotherapy and chemotherapy has been established in pre-clinical murine models, it has not been demonstrated in humans. Hyperpolarized ^{13}C MR metabolic imaging is a promising new technology that provides an increase in sensitivity over standard approaches of more than 10,000-fold and monitors changes in functional properties of the tumor rather than anatomic features[31-36]. Based on the results of the phase 1 clinical trial, the peak lactate/pyruvate ratio can be obtained from dynamic volume ^{13}C MR imaging data and provides the most robust biomarker of metabolism. Having successfully completed the first ever study with hyperpolarized ^{13}C pyruvate in patients with prostate cancer, our next step is to translate the technology to patients with other types of tumors and to use it to study treatment effects. Having recently developed radiofrequency coils and pulse sequences for detecting ^{13}C signals from the brain, we have now obtained the first hyperpolarized metabolic imaging data from patients with glioma. Our early studies have indicated that pyruvate crosses the blood brain barrier, that there is differential metabolism in normal brain versus tumor and that there is limited conversion of pyruvate to its metabolic products in regions that are stable following treatment. Understanding how the dynamic data obtained using hyperpolarized ^{13}C imaging relates can be combined with the more established steady state ^{1}H MRSI parameters and conventional anatomic imaging is critical for determining how to utilize the results obtained for making decisions about patient care.

7 STUDY OBJECTIVES AND PURPOSE

Objectives:

Primary:

1) To evaluate metabolic changes in the residual tumors in response to standard therapy in thirty patients with newly diagnosed glioblastoma (GBM). The hyperpolarized ^{13}C data and ^{1}H magnetic resonance spectroscopic imaging (MRSI) will be obtained after initial surgery and before receiving standard of care treatment with radiation therapy (RT) and temozolomide (TMZ) and at the first post-RT follow-up scan.

2) To examine metabolic markers of recurrent tumor and treatment related effects in patients with recurrent GBM. The hyperpolarized ^{13}C data and ^{1}H MRSI will be obtained from thirty patients with suspected recurrent GBM prior to surgery and used to define targets for tissue sampling. The histological and molecular characteristics of the samples will be evaluated and compared with corresponding metabolic parameters.

3) To assess the clinical value of using hyperpolarized ^{13}C metabolic imaging for detecting early response to standard therapy in patients with recurrent GBM. Thirty patients with suspected recurrent glioblastoma who are being treated with standard of care therapies will be studied prior to treatment (^{13}C + ^{1}H), at 7 days (^{13}C + ^{1}H) and 6-8 weeks after initial therapy (^{1}H only).

8 STUDY DESIGN & TREATMENT PLAN

8.1 Overall Study Design and Plan

This study will evaluate patients with GBM who receive infusion with hyperpolarized pyruvate (^{13}C) injection. The feasibility of using hyperpolarized ^{13}C MR metabolic imaging to reflect changes in pyruvate metabolism in response to therapy and differentiating recurrent tumor from treatment related effects will be assessed. Ninety adult patients with GBM will be recruited for this study.

Cohort 1 will comprise 30 patients with newly diagnosed GBM after initial surgical resection. They will receive two research MR imaging examinations that includes the acquisition of hyperpolarized ^{13}C metabolic data in combination with anatomic, diffusion, perfusion and lactate edited ^{1}H spectroscopic images before receiving standard of care treatment with RT and TMZ and at the first post-RT follow-up scan. The data from the pre-RT scan will be analyzed to evaluate the conversion of pyruvate to lactate and bicarbonate in normal appearing brain, the enhancing lesion, the non-enhancing lesion and the CNI lesion. The changes in ^{13}C parameters within these lesions between pre-RT and post-RT scans will be examined.

Cohort 2 will comprise 30 patients with suspected recurrent GBM who are going to receive surgery. Hyperpolarized ^{13}C data and 1H MRSI data will be obtained and used to define targets for tissue sampling. Ratios of lactate/pyruvate, conversion rates of pyruvate to lactate and bicarbonate, as well other ^{1}H imaging parameters will be compared with histological and molecular characteristics of the samples.

Cohort 3 will comprise 30 patients with suspected recurrent GBM who are going to be treated standard of care therapies. Patients in this cohort will undergo the research imaging examination prior to initiating therapy (baseline), at 7 days, and 6-8 weeks after the initiation of therapy. The changes in the lactate/pyruvate ratio, the CNI, the steady state lactate and other ^{1}H metrics will be evaluated to determine if they are associated with time to progression and overall survival.

Safety monitoring, including vital signs, serum biochemistry for function, and symptom monitoring will be performed at specified time points for up to 30 minutes after dosing is completed.

8.2 Benefit Assessment

The primary purposes of this study are to evaluate the clinical relevance of using this novel metabolic imaging in patients with GBM and to monitor changes in response to treatment for design in future studies. There are no direct benefits to individual subjects for participation in this study.

8.3 Study Timeframe

The total planned accrual for this trial is 90 subjects total. It is estimated that 2-4 subjects total will be enrolled per month. It is predicted that approximately 48 months will be required to complete accrual and data acquisition in this study. Long-term follow-up is required to investigate whether metabolic parameters from any of the three cohorts are associated with longer term clinical outcomes.

9 SELECTION AND WITHDRAWAL OF SUBJECTS

9.1 Procedures for Enrollment

Subjects enrolled in this study will be patients with glioma who have received a biopsy or surgical resection. Subjects will be reviewed for eligibility for this study during their new patient appointments or follow-up appointments by Dr. Chang and her colleagues in the Neuro-oncology Service at the UCSF Helen Diller Family Comprehensive Cancer Center (HDFCCC). Once it has been ascertained those 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.

9.2 Blinding

There will be no blinding in this study.

9.3 Eligibility Criteria

9.3.1 Inclusion Criteria

For Patients in Cohort 1: Histologically proven newly diagnosed GBM who will undergo standard of treatment with RT and TMZ.

For Patients in Cohort 2: Histologically proven recurrent suspected GBM who will receive surgical resection for the recurrence.

For Patients in Cohort 3: Histologically proven recurrent suspected GBM who will undergo standard treatment for the recurrence.

To be included in the study all subjects must also meet the following criteria:

(1) Patients must be > 18 years old and with a life expectancy > 16 weeks.
(2) Patients must have a Karnofsky performance status of ≥ 70.
(3) Patients must have adequate renal function (creatinine < 1.5 mg/dL) before imaging. These tests must be performed within 60 days prior to Hyperpolarized Imaging scan.
(4) Patients must not have any significant medical illnesses that in the investigator's opinion cannot be adequately controlled with appropriate therapy, would compromise the patient's ability to tolerate the imaging examination or any disease that will obscure toxicity or dangerously alter response to the imaging agent.
(5) Patients must not have New York Heart Association (NYHA) Grade II or greater congestive heart failure
(6) Patients must not have a history of myocardial infarction or unstable angina within 12 months prior to study enrollment.
(7) This study was designed to include women and minorities but was not designed to measure differences of intervention effects. Males and females will be recruited with no preference to gender. Minorities will actively be recruited to participate. No exclusion to this study will be based on race.

(8) Patients must sign an informed consent indicating that they are aware of the investigational nature of this study. Patients must sign an authorization for the release of their protected health information.
(9) Patients may not be known to be HIV-positive. HIV testing is not required for study participation.
(10) Patients must not have a history of any other cancer (except non-melanoma skin cancer or carcinoma in-situ of the cervix), unless in complete remission and off of all therapy for that disease for a minimum of 3 years.
(11) Patients must not be pregnant or breast feeding. Women of childbearing potential are required to obtain a negative pregnancy test within 14 days of Hyperpolarized Imaging scan. Effective contraception (men and women) must be used in subjects of child-bearing potential.

9.3.2 Exclusion Criteria

Subjects must be excluded from participating in this study if they are not able to comply with study and/or follow-up procedures.

9.4 Withdrawal and Termination Criteria

9.4.1 Subject Withdrawal

Subjects may be withdrawn for the following reasons:

Illness which prevents further administration of the IMP

Unacceptable toxicity/adverse events

Any other concerns regarding the health or well-being of the subject at the PI's (or designee's) discretion

Lack of co-operation on the part of the subject

Desire of the subject to cease participation

Failure of IMP to pass pre-administration testing.

If a subject is withdrawn after IMP administration, the date of and the reason for withdrawal must be entered into the CRF. If the withdrawal was due an adverse event, subject should be followed-up for outcome.

9.4.2 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.

Dosing may at any time be immediately suspended for safety reasons.

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

10 INVESTIGATIONAL MEDICINAL PRODUCT (IMP)

^{13}C is a stable, non-radioactive isotope of carbon. [1-^{13}C] pyruvate has 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 DNP. As [1-^{13}C] pyruvate has the same chemical characteristics as pyruvate and 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 [1-^{13}C] pyruvate and its metabolites, [1-^{13}C] alanine, [1-^{13}C]lactate, and [1-^{13}C]bicarbonate.

10.1 Formulation

The formulation of IMP in this study will contain 250 mM [1-^{13}C] pyruvate and up to 3 µM AH111501 sodium salt. The osmolality of this formulation is ~500 mosmol/kg. The IMP will be prepared adjacent to the MR suite and will be supplied as a ready-to-use solution for injection.

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.

10.2 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 will be responsible for receiving kit supplies, compounding the IMP, and performing drug accountability. See preparation methods (below) and appendix for more information.

10.3 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 (see Handling and Storage Safety Data Sheet in the Appendix) .

10.4 Preparation Methods

IMP will be compounded at the treatment unit i[redacted] with a pharmacist present to supervise compounding, and in accordance with the instructions provided by the manufacturer.

Briefly, the mixture of [1-^{13}C] pyruvic acid and AH111501 sodium salt is hyperpolarized by DNP at low temperature (~1 K) using dedicated instrumentation ("polarizer"). The polarizer is designed to be compatible with clinical use and incorporates an integral, disposable fluid path that contains all pharmaceuticals involved in the compounding process within a sterile environment throughout the polarization, dissolution and quality control (QC) processes[47]. After being polarized, the mixture of [1-^{13}C] pyruvic acid and AH111501 sodium salt is

dissolved in sterile water and forced through a filter consisting of a stack of polyethylene frits, which removes AH111501 to a level below 3 μM. The IMP is then collected in a receiver vessel and diluted with the TRIS/EDTA buffer solution. The yield is expected to be ~55 mL of 250 mM [1-^{13}C] pyruvate. The receiver, which is designed to accommodate the rapid QC processes, undergoes immediate QC testing to determine the pH, temperature, AH111501 concentration and polarization level. At the final step in the automated compounding procedure, the drug product is passed through a sterilizing filter (0.2 μm) within SpinLab immediately before entering the sterile Medrad syringe. Once the QC is complete, a sterile filter integrity test will be performed. This filter test will be performed at the same time as the IMP is collected in a Medrad syringe, transported to the MRI scanning room and setup on the power injector, thus not extending the time of injection. The filter integrity test is completed before the HP pyruvate dose can be released for patient use. Following approval for use from the pharmacist, the IMP is collected in a syringe and delivered to the MRI scanning room. The appropriate injection volume (per table 6) is then delivered to the subject at a rate of 3-5 ml/second. Note that for different shipments of [1-^{13}C] pyruvate, the concentration may be slightly different than 250 mM. The current concentration and corresponding injection volumes are provided (table 6). 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 very short life span of the Hyperpolarized Pyruvate (13C) precludes any sterility or bacterial endotoxin testing prior to administration. Sterility and bacterial endotoxin testing of a sample from the same material that is used for injection will be conducted after administration.

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 the appendix (Handling and Storage Safety Data Sheet).

10.5 Safety Information

Earlier studies of nonhyperpolarized pyruvate outside of the United States were summarized in section 5.5. In these studies, the following AEs were reported:

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 section 5.6.

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.

11 STUDY PROCEDURES

Table 1: Study Schedule of Events

	Pre-imaging	Imaging #1			Pre-imaging	Imaging #2[b]		
			Post-administration				Post-administration	
-	Screening	Baseline (prior to injection and imaging)	1-2 minutes after injection of IMP	Remaining duration of scan	Repeat screening prior to repeat imaging	Second injection[b] (8-12 weeks later) Baseline (prior to injection and imaging)	1-2 minutes after injection of IMP	Remaining duration of scan
Informed consent	X							
Demographics/ medical history	X							
Karnofsky performance status	X				X			
Imaging			X				X	
Limited physical exam	X				X			
Prior/con-comitant medication	X				X			
Vital signs[a]		X				X		
Blood for safety analysis [c]	X				X			
Adverse events				X				X
Injection site monitoring				X				X

[a]Systolic and diastolic blood pressure and heart rate; respiratory rate, body temperature and blood oxygen saturation only at baseline

[b]A window of +/- 1 week will be allowed for the repeat imaging.

[c]Creatinine lab testing within 60 days of study, and urine or serum pregnancy test within 14 days of study.

11.1 Schedule

11.1.1 Screening Period

Subjects will be screened within 90 days prior to IMP administration.

Signed and dated informed consent must be obtained from all subjects prior to any protocol-specific procedures being performed.

All subjects must satisfy all eligibility criteria.

Demographic data, relevant medical history and medication will be recorded.

Blood samples may be drawn (only if Cr and/or pregnancy labs are needed).

- Cr lab test within 60 days of ^{13}C injection/imaging
- Serum pregnancy test within 14 days of ^{13}C injection/imaging

Karnofsky performance status will be assessed.

A limited physical exam will be performed.

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.

11.1.2 Preparation Period

All subjects will be examined to ensure that they still satisfy all eligibility criteria. This will include repeat assessment of Karnofsky performance status, limited physical examination and medication history.

Immediately before the subject is moved into the MR suite, vital signs will be recorded.

11.1.3 Treatment Period

Each subject will receive a protocol-stipulated dose of IMP and will be imaged as described in Section 11.2. The injection site will be monitored throughout the scan for any abnormal findings. Monitoring of AEs will start from time of administration of IMP up to study completion.

11.1.4 Evaluation Prior to Second Imaging

Patients will undergo repeat screening by nurse or designated personnel to verify that they still meet eligibility criteria. This will include repeat assessment of Karnofsky performance status, vital signs, physical examination, medication history in addition to monitoring for adverse events and evaluation of the prior injection site. Patients will then undergo imaging (with associated procedures and monitoring) as per their first imaging.

Note that any patient who experiences grade 3 or higher toxicity on the first imaging exam which meets the following criteria:
1. not an asymptomatic laboratory abnormality
2. thought possibly to be related
3. clinically significant will not undergo repeat imaging per the second part of the study.

11.2 Procedures

Demographic Data
The subject's demographic data (gender, age, ethnicity and race, weight, height, and BMI) will be recorded at screening and recorded in the CRF.

Medical and Surgical History
The subject's relevant medical and surgical history (a condition that is either active at enrolment or adversely impacts the subject's condition at enrolment) will be recorded at screening and recorded in the CRF.

Prior and Concurrent Medication
Information about prior, concurrent, and procedural medications taken by a subject during the study period (from screening until the 1-day follow-up examination), including the indication will be collected from Apex or recorded in CRF.

Physical Examination
A limited physical exam will be performed at screening. This will include assessment for the presence of abnormalities in general appearance, lungs, cardiovascular system, and abdomen.

Vital Signs

Vital signs (systolic and diastolic blood pressure, heart rate, respiratory rate, body temperature, and blood oxygen saturation (by pulse oximetry) will be monitored at baseline (immediately before the subject is transferred to the MR suite).

Clinical Laboratory Evaluation

Blood may be drawn for analysis of adequate renal function (creatinine < 1.5 mg/dL). It is anticipated that the maximum amount of blood taken will not be more than 5 ml for all the samples taken during the subject's study participation.

Clinical laboratory parameters assessed in this study are displayed below.

Table 2 Clinical Laboratory Parameters

Creatinine
Serum or urine pregnancy test

Image Acquisition

The sample polarization will start approximately one hour prior to the proposed time for injection so that it will have time to build up. An IV will be set up prior to positioning the subject in the scanner to provide access for delivering the ^{13}C pyruvate. The coil set up will be either the ^{13}C clamshell transmit with the dual 4 channel ^{13}C receive coils, 32-channel receive array with integrated preamps and a high-power transmit coil or a new $^{13}C/^{1}H$ dual tuned head coil. Anatomical images will be acquired using gradient echo, fast spin echo or FLAIR sequences first in order to prescribe excitation location for ^{13}C dataset.

a) Anatomic imaging: a 3-plane localizer will be acquired to define the graphical prescription. This will be followed by axial FSE, gradient echo or FLARI images.
b) ^{13}C Calibration: the ^{13}C scan parameters (R1, R2, Ax, TG) will be calculated based on the external reference standard signal.
c) Test ^{13}C MRSI data: this will be obtained to make sure that the sequence and MR hardware components are functioning. It will show only the reference standard.
d) Dissolution: Given a successful test scan, the dissolution will be initiated and, if the sample passes the QC and sterile filter integrity test, it will be injected into the subject.
e) Hyperpolarized ^{13}C MRSI data: This will be acquired with a short delay after the end of the injection to allow for delivery of the pyruvate to the brain. For dynamic data the time resolution will be 3-5s. For the single time point data, the start time will be adjusted based upon when it is anticipated that the lactate/bicarbonate/pyruvate will be at a maximum.

Standard ^{1}H MRI and MRSI data will be obtained after the ^{13}C component of the examination has been completed.

Treatment Compliance

Subjects will receive the IMP under direct supervision of study personnel. The administration volume will be checked, and the kit component vial codes and volume administered will be recorded in each subject's CRF.

Injection Site Monitoring
Injection site monitoring will be performed throughout the study duration.

Abnormal injection site findings include, but are not limited to, extravasation, bleeding, hematoma, redness, and infection.

Monitoring of AEs
Symptoms will be monitored from just before IMP administration until the end of study participation. Study staff will ask subjects how they feel throughout the study duration. The subjects will be instructed to immediately report any symptoms and signs to the study staff (i.e., between formal observations).

11.3 Supportive Care

Subjects should receive full supportive care, including transfusions of blood and blood products, erythropoietin, antibiotics, antiemetics, etc., when appropriate (e.g., should any AEs occur).

The Principal Investigator or a designated physician (Robert Bok, MD) 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.

Study personnel will remain vigilant for the occurrence of AEs, particularly those that may be life threatening. Personnel trained in the acute management of anaphylaxis and other emergencies and who have access to appropriate clinical supplies will be immediately available for up to 30 minutes after dosing. Treatment of SAEs should be primarily supportive of vital functions.

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.

12 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

12.1 Adverse Event Definitions

12.1.1 Adverse Event

An adverse event (AE) is any unfavorable or unintended sign, symptom or illness occurring in a research subject, that develops or worsens during the study (treatment or post-treatment follow-up period), regardless of causality. This includes abnormal clinical or laboratory findings.

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.

12.1.2 Unexpected Adverse Event

An AE is unexpected if it is not listed in the current labeling for the investigational agent (Investigator's Brochure, Package Insert, etc.), whether or not it has been previously reported. An unexpected AE may also be one that, although listed in the labeling, is greater in severity or occurs more frequently than expected.

12.1.3 Serious Adverse Event

A serious adverse event (SAE) is any AE occurring at any dose that results in any of the following outcomes:

Death

Life-threatening (places the subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred (i.e., at risk of death at the time of the event); it does not refer to an event which hypothetically might have caused death if it were more severe)

Inpatient hospitalization or prolongs existing hospitalization

Persistent or significant disability/incapacity (a substantial disruption of a person's ability to conduct normal life functions)

Birth defect/congenital anomaly

Cancer

Any important medical event that may not result in prior listed outcomes but, based upon appropriate medical judgment, may jeopardize the subject, and may require medical or surgical intervention to prevent one of the prior listed outcomes.

12.2 AE Monitoring

The following data will be collected and evaluated for safety assessment:

Vital signs: systolic and diastolic blood pressure and heart rate at baseline

Injection site monitoring

Pre-treatment events

Post-treatment events (AEs and SAEs)

Additional diagnostic tests may be indicated to determine a more precise diagnosis of the subject's condition.

The principal investigator will conduct continuous review of data and subject safety at regularly held study group or site committee meetings, where study investigators and clinical research staff will update and review results since the prior meeting (subjects enrolled, AEs, dose adjustments, observed responses, and other study-related issues). The discussion will be documented in the "meeting minutes."

If the study demonstrates an increase of any Grade 3 or 4 AEs above the rate reported in the investigational brochure or package insert, this will be reported to the Data Safety Monitoring Committee (DSMC) at the time the increased rate is identified.

12.3 AE and SAE Reporting

AEs of all grades, regardless of attribution, will be recorded and updated at each study visit and will be entered into the CTMS in time for regular AE monitoring (see above).

AEs will be reported any applicable regulatory authorities within required timelines. The date at which the report(s) was submitted will be documented in the CTMS. Hard copies of the report will be maintained in the regulatory files.

AEs will also be reported as per local IRB requirements.

The UCSF protocol number and protocol-specific patient ID should be used for all reports. All reports should contain at minimum:

Whether it is expected or unexpected.

Investigator's assessment of causality.

The investigator also will conduct follow-up on adverse events, as required.

Any SAE occurring at baseline and until 7 days after IMP administration must be reported. SAEs occurring more than 7 days after IMP administration need only be reported if a relationship to the IMP is suspected.

SAE reporting will be in accordance with applicable federal regulations, institutional guidelines, and any other requirements. For reference:

UCSF IRB website for guidance in reporting serious adverse events:
http://www.research.ucsf.edu/CHR/Guide/Adverse_Events_Guidelines.asp

FDA website for guidance in reporting serious adverse events:
http://www.fda.gov/Safety/MedWatch/HowToReport/default.htm

MedWatch forms and information:
http://www.fda.gov/medwatch/getforms.htm

12.4 Reporting Pregnancy

The subject must be advised by the investigator to inform him/her immediately if they suspect their partner became pregnant within 1 month after the subject received IMP.

When a subject report a pregnancy (post-IMP administration) to the investigator, a pregnancy test should be arranged for the subject's partner by the investigator within 7 days of the pregnancy being reported.

12.5 Procedural Risks to subjects

The risks of drawing blood and placement of an infusion line include temporary discomfort from the needle stick, bruising at the puncture site, infection, and, very rarely, nerve damage.

There is no radiation exposure associated with administration of a MR scan.

13 DATA AND SAFETY MONITORING PLAN

In addition to the AE monitoring discussed in the previous section.

13.1 Oversight and Monitoring Plan

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

- Annual auditing
- Review of serious adverse events
- Minimum of biennial regulatory auditing

The UCSF HDFCCC Data and Safety Monitoring Committee (DSMC) is responsible for participant safety for all HDFCCC institutional clinical trials. Greater than minimal risk nontherapeutic studies are characterized as low risk studies due to the trial design, as there isn't administration of drugs or complementary therapy that puts the participants at significant risk.

13.2 Monitoring and Reporting Guidelines

Investigators will conduct a continuous review of data and participant safety at monthly site committee meetings where the status of each participant is discussed and documented in the site committee minutes.

For "greater than minimal risk" nontherapeutic trials, the assigned DSMC Senior Monitor/Auditor will audit three of the enrolled participants once per year, with a maximum of ten participant charts audited during the entire course of auditing this trial until IRB closure.

If blood or tissue banking trials are determined to be "greater than minimal risk", then only Serious Adverse Events (SAEs) recorded in OnCore will be reviewed at each DSMC meeting for these trials.

After completion of each auditing visit, the 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. An abbreviated regulatory review (i.e., reviewing protocol and consent versions, SAEs, PVs, DOA logs, 1572 forms, etc.) will occur at each participant monitoring review; however, a full regulatory review will occur on a biennially basis by the DSMC for regulatory compliance.

Auditing of all enrolled participants in these trials will be complete after 10 enrolled participants have been audited. However, regulatory reviews of the trial, safety reviews (i.e., Serious Adverse Event (SAE) reviews and Protocol Violation (PV) reviews), and audit/inspection preparation (as applicable) will continue until the trial is closed by the IRB.

13.3 Review and Oversight Requirements

Adverse Event Monitoring

All Grade 3-5 adverse events (AEs), whether or not considered expected or unexpected and whether or not considered associated with the study intervention or 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 study intervention or procedure. Attribution categories are:

- **Definite –** clearly related to the study intervention or procedure.
- **Probable –** likely related to study intervention or procedure.
- **Possible –** may be related to study intervention or procedure.
- **Unrelated –** clearly not related to the study intervention or procedure.

All clinically significant adverse events entered into OnCore® will be reviewed on a monthly basis at the Site Committee meetings

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 adverse experience*,
- Inpatient hospitalization or prolongation of existing hospitalization,
- Persistent or significant disability/incapacity,
- Congenital anomaly/birth defect, or cancer, or
- Any other 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 that changes the risk/benefit ratio of the study.

* A life-threatening adverse experience is any AE that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

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. 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 was sent to all required reporting agencies will be documented in OnCore.

If a death occurs during the treatment phase of the study and is determined to be possibly, probably, or definitely related either to the study intervention or procedure, the Investigator or his/her designee must notify the DSMC Chair or Vice Chair and DSMC Director within one business day.

Review of Adverse Event Rates

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 equirements.

Data and Safety Monitoring Committee Contacts:

DSMC Chair: Katie Kelley, MD
Phone
Email
Box
UCSF HDFCCC
San Francisco, CA 94158

DSMC Director: John McAdams, MS, CCRP
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14 SAFETY AND IMAGING VARIABLES

14.1 Study Endpoints

1) Measurements of Safety: Assessment of the occurrence of clinically significant changes in safety variables from baseline. Safety endpoints include monitoring for the occurrence of treatment-emergent AEs. Monitoring will occur to evaluate for dose-limiting toxicity. Toxicities will be graded using the National Cancer Institute (NCI) Common Terminology (Toxicity) Criteria for Adverse Events (CTCAE) version 4.0 and can be found at this link: http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf

2) The changes in ^{13}C parameters from baseline to the post-RT scan will be compared in the normal appearing brain, enhancing lesion, non-enhancing lesion and the choline-to-NAA index (CNI) lesion.

3) The ^{13}C metabolic parameters from the recurrent lesions will be compared to those in the regions of treatment related effects.

4) The ^{13}C and ^{1}H metabolic parameters will be evaluated to determine the association to time to progression and overall survival.

15 STATISTICAL METHODS AND PLANNED ANALYSIS

15.1 General Statistical Considerations

All subjects who are enrolled in the study and receive any amount of IMP will be included in the safety analysis. All available safety data will be used and will be analyzed using descriptive statistics.

Tabulations of summary statistics, graphical presentations, and statistical analyses will be performed using statistical software such as Stata® and Statistica®. All summary tables will be presented separately for each part of this pilot study. In addition, baseline parameters will also be presented for the entire study sample. The last pre-administration observation will be used as the baseline value for calculating post-administration changes from baseline.

15.2 Sample Size Determination and Analysis

For Cohort 1, we will use a repeated measures ANOVA as a global test for differences between ^{13}C metabolite parameters in the different ROIs. Given 30 patients and assuming sphericity, there would be 90% power to detect an effect size of 0.25, assuming $\alpha = 0.05$ and a 0.5 correlation among repeated measures. We would expect a difference at least this large to be clinically useful. We will assess the differences in the metabolite parameters between the 2 scans using a two-sided paired t-test or Wilcoxon signed rank test if the assumptions for the t-test are not met.

For cohort 2, we will first assess if the in vivo metabolite parameters are accurate at identifying recurrent tumor vs. gliosis/treatment effect as confirmed by histological analysis. Standard tests of significance will be used for paired samples to test the hypothesize that regions confirmed by histology and immunohistochemistry to correspond to recurrent GBM will have higher lactate/pyruvate ratios and higher CNI than samples from regions of treatment effect. A secondary, exploratory analysis will be in evaluating whether there are correlations between the in vivo metabolite parameters and the histology/IHC assays.

For cohort 3, we will compare the anatomic and metabolic lesion imaging values at 3 time points: baseline, 7 days post baseline, 6-8 weeks post baseline. We hypothesize that ratios of lactate/pyruvate will be decreased at the 7-day scan, and CNI intensities will be decreased at the 6-8 week scan relative to baseline. Standard survival analysis with Cox proportional hazards and Kaplan Meier methods will be employed. Because of the exploratory nature of this analysis, no consideration was made for power and effect size. The results will be used to define the potential effect size and will provide preliminary data for planning a larger study using this novel methodology.

15.3 Subject Characteristics

Demographic information (e.g., age, race, height, weight, and body mass index) will be summarized using descriptive statistics. Medical histories will be summarized by counts and percent. Concurrent medications will be recorded and coded using a standard classification system and grouped by primary and secondary classes, if a

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

15.4 Patient Replacement

Patients may be replaced for the following reasons:
Inability to tolerate/complete a baseline imaging scan (with no administration of IMP)
Failure of the IMP to pass testing, preventing its administration

Patients will not be replaced for toxicity.

In addition, patients may be replaced for failing to complete the second scan if secondary to reasons that are not thought to be study-related toxicity for that patient (e.g., scheduling or patient becomes ineligible at rescreening). No replacement will occur if this failure to complete the second scan is related to toxicity.

15.5 Early Stopping Rule for Safety

Toxicity will be reviewed on a continuous basis. If at any time (after two or more patients have been accrued) more than 33% of patients experience ≥grade 2 toxicity that is possibly, probably or definitely attributable to the IMP, (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. During that period of time, the performance of follow-up scans will also be halted for safety reasons.

15.6 Additional Safety Analysis

15.6.1 Vital Signs

For blood pressures and heart rate recorded after IMP administration, the following safety endpoints will be summarized for each part of the study:

The occurrence of changes from baseline, at each post-administration time point, greater than a pre-specified magnitude (20 mm Hg for systolic blood pressure, 10 mm Hg for diastolic blood pressure, 10 beats per minute for heart rate).

The occurrence of post-administration values outside the normal limits.

15.7 Significance Level

No formal statistical tests will be performed.

15.8 Procedures for Missing, Unused and Spurious Data

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

16 DATA COLLECTION AND MANAGEMENT

16.1 CRF and Source Documentation

Study data will be entered into OnCore (Online Collaborative Research Environment) via electronic case report forms (CRFs) by the clinical research coordinator (CRC). Single data entry will be used.

All source documentation should be kept in separate research folders for each patient.

All source documentation and OnCore data will be available for review/monitoring as needed.

The principal investigator will be responsible for ensuring the accurate capture of study data. At study completion, when the database has been declared to be complete and accurate, the database will be locked. Any changes to the database after that time can only be made by joint written agreement between the principal investigator, the statistician, and the protocol project manager.

16.2 Archiving

All study documentation will be archived for a minimum of 3 years following completion or discontinuation of the study (as recommended by the University of California Office of the President).

17 ETHICAL AND REGULATORY CONSIDERATIONS

17.1 Protocol Review Committee

This study will be reviewed by the UCSF Comprehensive Cancer Center Protocol Review Committee (PRC) for scientific merit. After initial review and approval, the study will be reviewed at least once a year for scientific progress. Any changes to the protocol are to be reviewed and approved by the PRC.

17.2 Institutional Review Board (IRB) / Independent Ethics Committee (IEC)

The study will be reviewed by the Committee on Human Research (CHR), UCSF's IRB. After initial review and approval, the study will be reviewed at least once a year as per FDA regulations (21 CFR 56.109). Any changes to the protocol and consent form are to be reviewed and approved by the CHR.

It is the responsibility of the Principal Investigator to keep the CHR informed of the progress of the study, including changes to the protocol or consent form, exceptions or deviations from the protocol, and any new developments which may affect subject safety or willingness to participate.

17.3 Investigational New Drug Application (IND)

An IND will be filed with the FDA. Annual reports will be submitted to the FDA within 60 days of the anniversary date that the IND went into effect, as per FDA regulations (21 CFR 312.33)

17.4 Conduct of the trial

The investigators will obtain informed consent and will conduct the trial in accordance with Federal regulations, institutional requirements, and the Declaration of Helsinki.

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19 APPENDICES

19.1 Information on Investigational and Registered Products

The reference document for this study is the Investigator's Brochure for Pyruvate Injection. The reference document provides up-to-date information on the efficacy and safety of the IMP, and is used for assessing expectedness of Serious Adverse Drug Reactions (ADRs), in order to determine regulatory reportability. An unexpected ADR is a reaction, for which the nature, seriousness, severity or outcome is not consistent with the applicable product information, e.g., the Investigator's Brochure for an unapproved IMP.

19.2 Cockcroft Gault Equation for Determining Creatinine Clearance

$$\frac{(140\text{-age})(\text{Weight in kg})}{(\text{Creatinine} \times 72)} = \text{estimated creatinine clearance (mL/min)}$$

19.3 Karnofsky Performance Status Scale

Table 3 Karnofsky Performance Status Scale

Grade	Description
100	Normal, no complaints, no evidence of disease
90	Able to carry on normal activity: minor symptoms of disease
80	Normal activity with effort: some symptoms of disease
70	Cares for self: unable to carry on normal activity or active work
60	Requires occasional assistance but is able to care for needs
50	Requires considerable assistance and frequent medical care
40	Disabled: requires special care and assistance
30	Severely disabled: hospitalization is indicated, death not imminent
20	Very sick, hospitalization necessary: active treatment necessary
10	Moribund, fatal processes progressing rapidly
0	Dead

19.4 Normal Limits for Vital Signs and EKG Variables

Table 4 Criteria for Normal Limits for Vital Signs*

	Normal Limits	
Vital Signs Parameter	**Low**	**High**
Systolic Blood Pressure (mmHg)	85	139
Diastolic Blood Pressure (mmHg)	60	89
Heart Rate (bpm)	60	100
Respiration Rate (rpm)	12	22
Body Temperature	36.4 C 97.5 F	37.7 C 99.5 F
Oxygen Saturation (%)	93	100
Body Weight (kg)	41	113
Body Mass Index (kg/m^2) [a]	18.5	24.9

bpm = beats per minute; rpm = respirations per minute.

* These normal limits are for purposes such as making data comparisons,

but not for determining eligibility or defining adverse events.

[a] Staff at UCSF will establish whether potential subjects comply with the BMI requirements stipulated under exclusion criterion 6.

Table 5 Criteria for Normal Limits for EKG Variables

EKG Variable	Normal Limits (ms)	
	Low	High
PR interval	120	200
QRS interval	50	100
RR interval	600	1000

19.5 Dosing Table

Table 6 Injection Volumes (234 mM [1-^{13}C] pyruvate) for Individual Body Weights (mL)

Body Weight (kg)	0.07 ml/kg	0.14 ml/kg	0.28 ml/kg	0.36 ml/kg	0.43 ml/kg
40	3	6	12	15	18
41	3	6	12	16	19
42	3	6	13	16	19
43	3	6	13	16	19
44	3	6	13	17	20
45	3	6	14	17	20
46	3	6	14	18	21
47	3	7	14	18	21
48	3	7	14	18	22
49	3	7	15	19	22
50	4	7	15	19	24
51	4	7	15	19	24
52	4	7	16	20	24
53	4	7	16	20	25
54	4	9	16	20	25
55	4	9	16	21	26
56	4	9	17	21	26
57	4	9	17	22	27
58	4	9	17	22	27
59	4	9	18	22	27
60	4	9	18	24	28
61	4	10	18	24	28
62	4	10	18	24	29
63	4	10	19	25	29
64	4	10	19	25	30
65	5	10	19	25	30
66	5	10	19	26	30
67	5	10	20	26	31
68	5	11	20	26	31
69	5	11	20	27	32
70	5	11	21	27	32

71	5	11	21	28	33
72	5	11	21	28	33
73	5	11	21	28	33
74	5	11	22	29	34
75	5	12	22	29	34
76	5	12	22	29	35
77	5	12	24	30	35
78	5	12	24	30	36
79	6	12	24	30	36
80	6	12	24	31	36
81	6	12	25	31	37
82	6	12	25	32	37
83	6	13	25	32	38
84	6	13	26	32	38
85	6	13	26	33	40
86	6	13	26	33	40
87	6	13	26	33	40
88	6	13	27	34	41
89	6	13	27	34	41
90	6	14	27	34	42
91	6	14	27	35	42
92	6	14	28	35	43
93	7	14	28	35	43
94	7	14	28	36	43
95	7	14	29	36	44
96	7	14	29	37	44
97	7	15	29	37	45
98	7	15	29	37	45
99	7	15	30	38	46
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101	7	15	30	38	46
102	7	15	31	40	47
103	7	15	31	40	47
104	7	16	31	40	48
105	7	16	31	41	48
106	7	16	32	41	49
107	7	16	32	42	49
108	9	16	32	42	49
109	9	16	33	42	50
110	9	16	33	43	50
111	9	17	33	43	51
112	9	17	33	43	51
113	9	17	34	44	52
114	9	17	34	44	52
115	9	17	34	44	52
116	9	17	34	45	53

117	9	17	35	45	53
118	9	18	35	45	54
119	9	18	35	46	54
120	9	18	36	46	56
121	9	18	36	47	56
122	10	18	36	47	56
123	10	18	36	47	57
124	10	18	37	48	57
125	10	19	37	48	58
126	10	19	37	48	58
127	10	19	38	49	59
128	10	19	38	49	59
129	10	19	38	49	59
130	10	19	38	50	60
131	10	19	40	50	60
132	10	19	40	51	61
133	10	20	40	51	61
134	10	20	41	51	62
135	10	20	41	52	62
136	11	20	41	52	62
137	11	20	41	52	63
138	11	20	42	53	63
139	11	20	42	53	64
140	11	21	42	53	64
141	11	21	42	54	65
142	11	21	43	54	65
143	11	21	43	54	65
144	11	21	43	56	66
145	11	21	44	56	66
146	11	21	44	57	67
147	11	22	44	57	67
148	11	22	44	57	68
149	11	22	45	58	68
150	12	22	45	58	69

19.6 Safety Data Sheets

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

19.6.1 Chemical product and company identification

Product name: Hyperpolarized Pyruvate-1- ^{13}C Solution for Injection.
Synonyms: Hyperpolarized Pyruvate (^{13}C) 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: [redacted]

19.6.2 Composition and information on ingredients

Composition: Pyruvic acid (AH110896 and AH111710), $C_3H_4O_3$ AH110896 is the 13-C enriched pyruvic acid; [1-^{13}C] pyruvic acid. AH111710 is pyruvic acid (no enrichment). AH111501 sodium salt (EPA), $Na_3C_{64}H_{87}O_{18}S_{12}$ Trometamol ($C_4H_{11}NO_3$), Na_2EDTA ($C_{10}H_{14}N_2Na_2O_8$), 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, Na_2EDTA is added as a metal complexing agent, NaOH will provide a pH of 6-8 when mixed with the pyruvic acid.
Main ingredients: Tromethamol ($C_4H_{11}NO_3$) CAS No. 77-86-1 Na_2EDTA ($C_{10}H_{14}N_2Na_2O_8$) CAS No. 6381-92-6 NaOH CAS No.1310-73-2 Pyruvic acid ($C_3H_4O_3$) CAS No.127-17-3

19.6.3 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

19.6.4 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

19.6.5 Fire-fighting measures

Flammability: Non-flammable
Fire Extinguishing Media: H_2O, CO_2 , or dry powder
Fire Fighting Procedures: Not known
Decomposition and Explosion Hazards: Not known

19.6.6 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

19.6.7 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 air-tight container.

19.6.8 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).

19.6.9 Physical and chemical properties

Appearance, color: colorless to slightly colored, clear liquid	
Odour: No characteristic odour	
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

19.6.10 Stability and reactivity

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