A Pilot Study to Assess lactate and bicarbonate detection within Malignant Brain Tumors using [1-^{13}C]-pyruvate DNP Magnetic Resonance Spectroscopy (MRS)

Study Protocol and Statistical Analysis Plan

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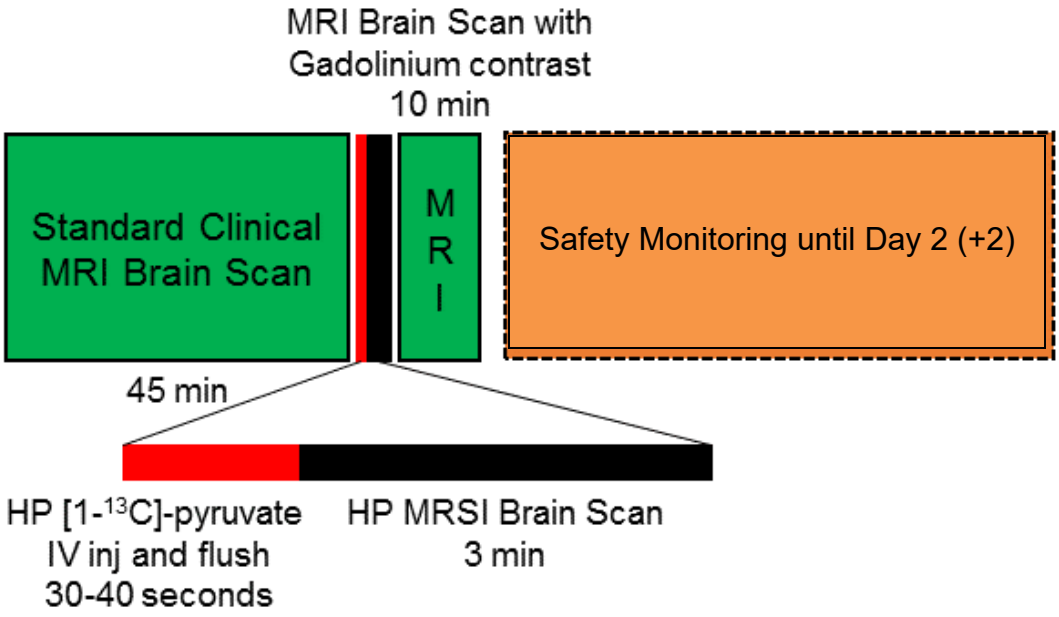
SCHEMA


MRI Brain Scan with
Gadolinium contrast
10 min
Standard Clinical
MRI Brain Scan
M
R
I
Safety Monitoring until Day 2 (+2)
45 min
HP [1-13C]-pyruvate
IV inj and flush
30-40 seconds
HP MRSI Brain Scan
3 min

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse event
ALT	Alanine aminotransferase
AST	Asparagine aminotransferase
BIC	Bicarbonate
CBV	Cerebral blood volume
CNS	Central nervous system
CRF	Case report form
DCA	Dichloroacetate
DLT	Dose Limiting Toxicity
DNP	Dynamic nuclear polarization
DSMC	Data Safety Monitoring Committee
ECG	Electrocardiogram
EPA	Electron paramagnetic agent
FDA	Food and Drug Administration
GBM	Glioblastoma multiforme
GCP	Good clinical practices
GFR	Glomerular filtration rate
GGT	Gamma-glutamyltransferase
GLY	Glycolysis
IRB	Institutional Review Board
IV	Intravenous
LAC	Lactate
MRI	Magnetic resonance imaging
MRSI	Magnetic resonance spectroscopy imaging
MRS	Magnetic resonance spectroscopy
OXPHOS	Oxidative phosphorylation
PD	Protocol Director
PYR	pyruvate
SAE	Serious adverse event
SNR	Signal to noise ratio
SRC	Scientific Review Committee
T1	Time constant for magnetization decay
ULN	Upper limit of normal
VS	Vital signs

1. OBJECTIVES

1.1. **Primary Objectives**

To assess the safety of IV injection of hyperpolarized [1-^{13}C]-pyruvate for MRI.

1.2. **Exploratory Objectives**

To assess the frequency and sensitivity with which lactate and bicarbonate signals can be detected in malignant brain tumors after IV injection of hyperpolarized [1-^{13}C]-pyruvate.

2. BACKGROUND

2.1. **Study Disease**

Our ultimate goal is to develop a strategy to manage cancer as a chronic disease for which treatment can be calibrated according to the tumor's metabolic state. In particular, cancer cells, in addition to their high growth rate, universally demonstrate an abnormal metabolism characterized by an overproduction of lactate (via a process known as glycolysis [GLY]) relative to the more energy efficient conversion of glucose to carbon dioxide and water (oxidative phosphorylation [OXPHOS]). Originally described over 80 years ago by Otto Warburg, this altered metabolism, more recently termed *metabolic reprogramming* [1], is now viewed as a hallmark of cancer and appears linked to the need for carbon skeletons to produce biomass and is thus intimately tied to the ability of cancer cells to proliferate [2]. The novel clinical implication of these observations suggest that a potential anti-tumor strategy lies in reversing the "Warburg Effect" by forcing cancer cell metabolism away from GLY towards OXPHOS. Although this would not result in cell kill *per se*, it should result in stabilization of growth with minimal toxicity. Although a number of targets exist for which active drugs could be directed, a major obstacle towards taking this to the clinic has been the *inability to measure cancer metabolism in vivo*.

Using a rat model, we have developed magnetic resonance spectroscopic imaging (MRSI) of hyperpolarized [1-^{13}C]-pyruvate as a noninvasive measure of lactate (Lac) and bicarbonate (Bic) production reflecting GLY and OXPHOS respectively [3], [4]. We now wish to translate this imaging technology to the clinic and demonstrate that these critical aspects of glucose metabolism can be measured in patients with glioma. A hyperpolarizer that can be used clinically has been purchased and is now ready to be utilized. We have funds in which to test 15 subjects and would like to perform a pilot test to confirm two necessary aspects of our hypothesis: (i) determining that our methods for polarizing [1-^{13}C]-pyruvate are safe for patient use; and (ii) evaluating if and how frequently we can detect lactate and bicarbonate signaling within brain tumor tissue.

2.2. **Study Agent/Device/Imaging procedure**

Dynamic nuclear polarization (DNP) magnetic resonance spectroscopic imaging (MRSI) is based on the transfer of polarization from the electron spins of paramagnetic centers embedded in a glassy frozen solution to neighboring nuclear spins through dipolar interactions, thus driving nuclei temporarily into a significant redistribution of the ordinary populations of energy levels [5]. These spin states are not stable in the sense that the induced massive spin polarization decays during a relatively short period but within this period allows one to follow a nucleus (usually carbon or nitrogen) as it is processed by a tissue. The absence of ionizing radiation and convenient integration with standard MRI imaging of hyperpolarized nuclei therefore offers the prospect of monitoring tumor metabolism noninvasively, the potential value of which depends on three factors: (i) the T1 signal needs to be sufficiently long so that enough material is preserved after injection to measure; (ii) the metabolic pathway itself much be sufficiently rapid so that the fate of the tracer can be measured; and (iii) the process must be relevant to tumor biology [6].

[1-^{13}C]-pyruvate is currently the tracer for which the most preclinical and clinical experience exists. Pyruvate (Pyr) is situated at a hub of the glucose utilization pathway where it can be reduced to lactate, amidated to alanine or catalyzed to acetyl CoA and CO_2. Although an issue existed early in its development as to whether it could cross the blood brain barrier fast enough to allow imaging in brain tumors without disruption, unequivocal imaging of brain tumor metabolism of hyperpolarized [1-^{13}C]-pyruvate in brain has been demonstrated [4].

Signal to noise (SNR) ratios are particularly strong for visualizing lactate (Lac), on which most studies to date have focused. Using this technology, it has been possible to show lactate dehydrogenase catalyzed interconversion of label between Pyr and Lac in several tumor types including GBM and that changes occur in the rate of labeling with treatment [4], [7]-[9]. Furthermore, it has also been demonstrated that an increased conversion of Pyr conversion to alanine is an early feature of experimental myc-driven hepatic tumors [10].

Based on numerous previous reports, tracing Pyr fate into the mitochondria has been more challenging and there have been numerous reports noting that imaging both lactate and bicarbonate (Bic) is difficult, especially in brain tumor [11], [12], creating barriers for using this imaging modality to explore the question of whether forcing OXPHOS slows cancer growth.

We recently overcame this hurdle through optimization of the acquisition protocol for spiral chemical shift imaging so as to be able to measure both lactate and bicarbonate in transplanted C6 glioma. Furthermore, we were able to demonstrate a consistent and marked decrease in the ratio of Lac/Bic acutely after the administration of DCA [8], substantiating at least that change in Pyr flux is occurring so that more substrate is directed into the mitochondria (since that is where Bic is generated).

This technology has been recently applied to clinical populations. The first-in-man Phase I trial examined a cohort of prostate cancer patients. It demonstrated the safety of administering 250 mM hyperpolarized [1-^{13}C]-pyruvate up to doses of 0.43 mL/kg body weight and that images could be acquired that demonstrated conversion of hyperpolarized pyruvate to lactate in regions of biopsy-proven tumor [13]. Since these experiments were reported from UCSF, this technology has become available for clinical study at Memorial Sloan Kettering Cancer Center, University of Texas at Southwestern (UTSW), Cambridge University, Oxford University, University of Toronto, and several other centers (including ours) are gearing up for study.

2.3. **Clinicaltrials.gov**

This study is submitted to IND-[redacted] for the IV injection of [1-^{13}C]-pyruvate, and will include submission of this protocol.

For clinicaltrials.gov compliance: This is a phase 0 (early phase 1), but relies in part on NIH funding so is required to register on clinicaltrials.gov and report results. For Clinicaltrials.gov results reporting, the following outcome measures will be used:

Primary Outcome Measure

- Title: Safety of hyperpolarized [1-^{13}C]-pyruvate injection
- Time Frame: Up to Day 4
- Description: Results will be reported as the number of Grade 2 or higher adverse events judged by the investigator to be clinically significant AND related/possibly-related to hyperpolarized [1-^{13}C]-pyruvate injection across all subjects administered study agent.

Exploratory Outcome Measure

- Title: Feasibility of Lactate and Bicarbonate signal detection
- Time Frame: Up to 4 days
- Description: Results will be reported as the percentage of subjects with CNS malignancy administered study agent and imaged who show ^{13}C MR signal detection above background noise level, reported separately for lactate and bicarbonate signals.

2.4. **Rationale**

See Section 2.1

2.5. **Preliminary results**

The proposed study will be the first time at Stanford where this technology will be used in humans. Prior use in cancer imaging, conducted at UCSF, is described in the Investigator's Brochure.

2.6. **Study Design**

This is an open-label, two-armed study of 5 healthy volunteers and 10 patients with known CNS malignancy.

- Arm 1: n = 5 healthy volunteers
- Arm 2: n = 10 patients with known CNS malignancy

3. PARTICIPANT SELECTION AND ENROLLMENT PROCEDURES

3.1. **Inclusion Criteria**

3.1.1. (Arm 2 patients with known CNS malignancy only) Patients with a known diagnosis of CNS malignancy, including metastases, with known enhancement on MR who are otherwise eligible to undergo MRI.
3.1.2. At least 18 years old.
3.1.3. eGFR > 30 ml/min.
3.1.4. (Arm 2 patients with known CNS malignancy only) No allergy to gadolinium.
3.1.5. Ability to understand and the willingness to sign a written informed consent document.

3.2. **Exclusion Criteria**

3.2.1. Refusal to have an IV placed for injection.
3.2.2. Acute major illness (e.g., unstable angina, etc.) or other condition that makes participation unsafe, per the investigator's judgement.
3.2.3. Total Bilirubin > 1.5 x ULN; AST/ALT > 2.5 x ULN; GGT > 2.5 x ULN.
3.2.4. Pregnant or breast-feeding.
3.2.5. Cardiovascular risk, including:
- Poorly controlled hypertension, defined as either systolic > 170 or diastolic > 110.
- Congestive heart failure
- Myocardial infarction within the past year
- QT prolongation, defined as pretreatment QTc > 440 msec in males or > 460 msec in females

3.2.6. (Arm 1 Healthy volunteers only) Undergoing active treatment for malignancy

3.3. **Informed Consent Process**

All participants must be provided a consent form describing the study with sufficient information for participants to make an informed decision regarding their participation. Participants must sign the IRB approved informed consent 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.

3.4. **Study Timeline**

3.4.1. Primary Completion

The study will reach primary completion once the last subject undergoes Day 2 safety evaluations.

3.4.2. Study Completion

We estimate that this study will take one year from first subject enrollment to complete.

4. IMAGING AGENT/DEVICE/PROCEDURE INFORMATION

4.1. **Hyperpolarized [1-^{13}C]-Pyruvate**

^{13}C is a stable non-radioactive isotope of carbon. [1-^{13}C]-pyruvate has exactly the same chemical characteristics as pyruvate. In [1-^{13}C]-pyruvate, the C-1 carbonyl has been enriched with ^{13}C-nuclei, which has a magnetic moment and can be hyperpolarized by DNP in the presence of an electron paramagnetic agent (EPA), i.e., AH111501 sodium salt (a stable trityl radical). Although [1-^{13}C]-pyruvate has the same chemical characteristics as unlabeled pyruvate and is metabolized the same way, 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 metabolic products.

Hyperpolarized [1-^{13}C]-pyruvate will be prepared by qualified staff at the Lucas Center under aseptic GMP/GLP conditions using a DNP polarizer (SpinLab, GE Healthcare). The imaging agent will be administered by IV injection at a rate of 5 ml/sec followed by a 20 ml saline flush at 5 ml/sec (on average 30-40 seconds). The formulation procedures are detailed in the current Investigator's Brochure (IB).

4.2. **Dissolution DNP MRSI**

Hyperpolarized [1-^{13}C]-pyruvate will be injected at a dosage of 0.43 ± 0.05 mL/kg body weight (concentration of 250 mM), the maximum safely administered dose attained in the Phase I study [13]. After hyperpolarization in the SpinLab polarizer, a syringe is automatically filled. The quality control module of the SpinLab measures the pyruvate concentration, volume, temperature, pH, polarization, and removal of the EPA in the syringe. A quality control assessment is then performed to ensure all parameters are reached, after which the syringe is released by the protocol director and immediately passed to a nurse or radiology technician for initiation of injection.

The overall imaging protocol will consist of 1) standard non-contrast clinical collection setup for brain imaging (~45 min), 2) hyperpolarized [1-^{13}C]-pyruvate MRSI (single- or multi-slice acquisition, ~3-min acquisition), and 3) standard clinical brain gadolinium-enhanced MRI (~10min).

4.3. **Dose Modification**

The starting dose of [1-^{13}C]-pyruvate will be 0.43 ± 0.05 mL/kg body weight (250 mM [1-^{13}C]-pyruvate). If we encounter a DLT at this dose (which seems unlikely since it has been well tolerated in safety studies elsewhere), we will decrease dose to 0.28 mL/kg.

The choice whether or not to use the [1-^{13}C]-pyruvate and/or gadolinium MRI contrast agent is made at Protocol Director's discretion. If the investigator choses to forego gadolinium contrast (due to concerns of gadolinium accumulation or other concerns), the second MRI brain scan may be skipped.

5. STUDY PROCEDURES

Healthy volunteers (Arm 1 of the study) will be recruited via an IRB-approved e-mail that refers them to the clinical research coordinator or research team. Eligible patients for arm 2 will be invited to participate at any time during their course of CNS malignancy treatment. Generally, arm 2 patients will be recruited during a regular clinic visit.

If the patient signs the informed consent form and agrees to participate, the following screening procedures will be performed within 14 days prior to Day 1:

- Collect demographics
- Measure vital signs (heart rate, temperature, blood pressure), height and weight
- Serum chemistry blood test
- Serum pregnancy test, if woman of childbearing potential
- Electrocardiogram (ECG)

On Day 1, MRI and MRSI will be performed in the Richard M. Lucas Center for Imaging at Stanford University. Production of hyperpolarized [1-^{13}C]-pyruvate will be coordinated with imaging schedule such that MRSI begins within 1 minute of hyperpolarized [1-^{13}C]-pyruvate administration. Each subject will undergo the following procedures:

- Measure vital signs
- MRI scan of brain, 45 min acquisition time using standard non-contrast clinical brain collection setup
- Administration of hyperpolarized [1-^{13}C]-pyruvate (250 mM, 0.43 ± 0.05 mL/kg body weight) IV injection at a rate of 5 mL/sec followed by a 20 mL saline flush at 5 mL/sec (on average 30-40 seconds)
- MRSI scan of brain, 3 min acquisition on a 3T MRI scanner equipped with both 1HF and ^{13}C RF coils.
- MRI scan of brain, 10 min acquisition time using standard Gadolinium-enhanced contrast clinical brain collection. Gadolinium contrast agent may or may not be administered at the discretion of the Protocol Director. Gadolinium will not be used for Arm 1 Healthy Volunteers.
- Within 10 min after the conclusion of image collection:
    - Measure vital signs
    - Collect any adverse events and monitor injection site
- At least once more 20-45 minutes after the conclusion of image collection:
    - Measure vital signs
    - Collect any adverse events and monitor injection site
- 1 to 5 hours post pyruvate administration:
    - Measure vital signs
    - ECG
    - Collect any adverse events and monitor injection site

Between Day 2 and 4, subjects will be contacted by phone or at an in-person clinic visit to collect any adverse events.

Once initial imaging is completed, patients will be followed as expected by standard of care. Patients are free to enroll in other studies.

6. STUDY CALENDAR

	Pre-Study (Within 14 Days of Day 1)	Day 1	Day 2 (+2 days)
Informed consent	X		
Demographics	X		
Vital signs	X	X[f]	
Height	X		
Weight	X		
Serum chemistry [a]	X		
ECG	X	X [e]	
Pregnancy test	X [b]		
Non-contrast enhanced MRI [c]		X	
[1-^{13}C]-pyruvate IV injection		X	
MRSI		X	
Contrast enhanced MRI [d]		X	
AE evaluation		X	X

a: GFR., Total bilirubin, SGOT[AST], SGPT[ALT], GGT.
b: Women of childbearing potential only
c: Before [1-^{13}C]-pyruvate IV injection and MRSI
d: After [1-^{13}C]-pyruvate IV injection and MRSI. Arm 1 Healthy Volunteers will not receive contrast
e: Within 1-5 hours post [1-^{13}C]-pyruvate IV injection
f: Collect on Day 1: at baseline prior to non-contrast MRI; within 10 min after the conclusion of the image collection, 20-45 min after the conclusion of image collection; and 1-5 hours post [1-^{13}C]-pyruvate IV injection

7. ADVERSE EVENTS AND REPORTING PROCEDURES

7.1. **Potential Adverse Events**

In the original Phase I studies with [1-^{13}C]-pyruvate, no dose-limiting toxicities or toxicities deemed to be clinically significant were noted [13]. We will consider any Grade 2 or higher toxicity judged related or possibly-related to [1-^{13}C]-pyruvate as significant (excluding asymptomatic lab abnormalities).

7.1.1. Stopping Rules

The study will be stopped if infection attributable to any component of the pyruvate infusion is observed. The study may resume after the necessary chemistry, manufacturing and controls (CMC) changes are made and the IND amendment is submitted to FDA and approved by IRB. In addition, the study will pause enrollment pending a safety review if two dose-limiting toxicity events (any Grade 2 or higher toxicity judged related or possibly-related to [1-^{13}C]-pyruvate, excluding asymptomatic lab abnormalities) are observed.

7.2. **Adverse Event Collection**

Adverse events will be graded according to CTCAE v5.0. Both Serious and Non-Serious Adverse Events will be clearly noted in source documentation and listed on study specific Case Report Forms (CRFs). The Protocol Director (PD) or designee will assess each Adverse Event (AE) to determine whether it is unexpected according to the Informed Consent, Protocol Document, or Investigator's Brochures, and related to the investigation.

All AEs, regardless of seriousness or relationship to investigational agent, will be collected from Day 1 through safety follow-up contact. From signing of informed consent to prior to Day 1, only AEs deemed related to study procedures will be collected. All Serious Adverse Events (SAEs) will be tracked until resolution or 4 days after the last dose of the study treatment. After the safety follow-up contact, any SAEs spontaneously reported to investigator and deemed related to investigational agent should be reported to the IND Sponsor-Investigator (Daniel Spielman, PhD).

7.3. **Adverse Event Reporting**

Non serious adverse events will be reported annually to FDA via an Annual Report ([redacted]) and to IRB via Continuing Review.

The PD (aka Principle Investigator) must immediately report within 24 hours of knowledge of event to the IND Sponsor-Investigator (Daniel Spielman, PhD), any serious adverse event, whether or not considered related to study drug. SAEs of all grades will be reported to the Sponsor-Investigator using the Stanford Cancer Institute SAE CRF.

Adverse events deemed serious, unexpected (i.e. not described in the protocol, Investigator's Brochure or informed consent documents) and related to investigational agent must be reported to the IND Sponsor-Investigator by email or fax using the FDA MedWatch form 3500a within 24 hours of knowledge of event. The IND Sponsor-Investigator is responsible for deciding whether the event meets IND Safety Reporting criteria.

Events will be submitted to Sponsor-Investigator Dr. Daniel Spielman, at:
Daniel Spielman, PhD
Professor of Radiology and Electrical Engineering (by courtesy)
1201 Welch Rd
Lucas Center, [redacted]
Stanford, CA 94305-5488

Phone: 650-723-8697
Fax: 650-723-9222
spielman@stanford.edu

It will be the responsibility of the Sponsor-Investigator to provide to the FDA all information concerning significant hazards, contraindications, side effects or precautions felt significant to the safety of the investigational agent being studied. The PD is responsible for accurate and timely communication to the Sponsor-Investigator of any events reportable to FDA.

SAEs CTCAE Grade 2 and above, and all subsequent follow-up reports will be reported to the Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) using the study specific CRF regardless of the event's relatedness to the investigation. Following review by the DSMC, events meeting the IRB definition of 'Unanticipated Problem' will be reported to the IRB using eProtocol within 10 working days of DSMC review, or within 5 working days for deaths or life-threatening experiences.

8. REGULATORY CONSIDERATIONS

8.1. **Institutional Review of Protocol**

The protocol, the proposed informed consent and all forms of participant information related to the study (e.g. advertisements used to recruit participants) will be reviewed and approved by the Stanford IRB and Scientific Review Committee (SRC). Any changes made to the protocol will be submitted as a modification and will be approved by the IRB, and SRC if applicable, prior to implementation. The Protocol Director will disseminate the protocol amendment information to all participating investigators.

Protocol amendments will be submitted to the FDA under IND [redacted] in accordance to the regulations.

8.2. **Data Management Plan**

The Protocol Director, 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 treatment outcomes for data analysis. Case report forms will be developed using the Oncore database system and will be maintained by CCTO. CRFs will be kept in a locked office, only accessible to the research team."

8.3. **Data and Safety Monitoring Plan**

During the clinical investigation, the Protocol Director will evaluate the progress of the trial, including periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of trial sites, and other factors that can affect study outcome. Monitoring of the trial will occur every 8 weeks and a record of monitoring activities will be maintained by the study team.

The Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) will audit study related activities at least annually in accordance with the DSMC SOP to determine whether the study has been conducted in accordance with the protocol, local standard operating procedures, FDA regulations, and Good Clinical Practice (GCP). This may include review of regulatory binders, case report forms, eligibility checklists, and source documents. In addition, the DSMC will regularly review serious adverse events and protocol deviations associated with the research to ensure the protection of human subjects. Results of DSMC audits will be

communicated to the IRB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as needed.

9. MEASUREMENTS

9.1. **Primary and Exploratory Outcome measures**

Primary Outcome Measure: This is a pilot study which will allow us to develop a novel MRS technique for clinical application. Our primary measurement will be safety of [1-^{13}C]-pyruvate IV injection defined as the absence of Grade 2 toxicity (except for asymptomatic lab increases).

Exploratory Outcome Measure: Detection of lactate and bicarbonate production in tumor and normal brain tissue, defined as the frequency of ^{13}C MR signal detection above background noise level of both lactate and bicarbonate in all tumors screened.

9.2. **Measurement Methods**

Images will be analyzed for several conventional MR parameters, including tumor volume and CBV. Pyruvate, bicarbonate and lactate signals will be assessed by peak integration of the corresponding spectral peaks.

9.3. **Measurement Time Points**

Assessments will be made only at one time point.

9.4. **Response Review**

No response review is planned.

10. STATISTICAL CONSIDERATIONS

10.1. **Statistical Design**

This pilot study seeks to demonstrate safety of the [1-^{13}C]-pyruvate IV injection as well as the ability to detect lactate and bicarbonate spectra. Safety will be defined as the absence of Grade 2 toxicity (except for asymptomatic laboratory abnormalities).

Another goal is an assessment of whether lactate and bicarbonate spectra can be detected in tumor. Three answers are anticipated. Either there will be bicarbonate spectra detected in all, some or none. Our experimental data suggests that we should be able to see it in all enhancing areas, although the technical differences between experimental and clinical machines does not guarantee this outcome.

5 healthy volunteers and 10 patients with CNS malignancies will be assessed, which is the maximum that our budget will allow. These results will then be used to properly formulate a more formal experiment that will subsequently be submitted.

10.2. **Randomization**

No randomization.

10.3. **Interim analyses**

No interim analysis is planned.

10.4. **Descriptive Statistics and Exploratory Data Analysis**

N/A

10.5. Primary Analysis

N/A

10.6. Sample Size

Our budget allows 5 healthy volunteers and 10 patients with CNS malignancies for a total of 15 subjects. This number will allow us to accrue enough data to better understand lactate and bicarbonate detectability and safety.

10.7. Accrual estimates

15 subjects will be assessed over one year.

10.8. Criteria for future studies

The data obtained from the current study will be instrumental in helping us craft the first in human study assessing [1-^{13}C]-pyruvate DNP MRS in GBM patients receiving bevacizumab.

11. REFERENCES

[1] E. M. Palsson-McDermott and L. A. J. O'Neill, "The Warburg effect then and now: From cancer to inflammatory diseases," *BioEssays*, vol. 35, no. 11, pp. 965–973, Sep. 2013.

[2] M. G. Vander Heiden, L. C. Cantley, and C. B. Thompson, "Understanding the Warburg Effect: The Metabolic Requirements of Cell Proliferation," *Science*, vol. 324, no. 5930, pp. 1029–1033, May 2009.

[3] J. M. Park, S. Josan, T. Grafendorfer, Y.-F. Yen, R. E. Hurd, D. M. Spielman, and D. Mayer, "Measuring mitochondrial metabolism in rat brain in vivo using MR Spectroscopy of hyperpolarized [2-^{13}C]pyruvate.," *NMR Biomed.*, vol. 26, no. 10, pp. 1197–1203, Oct. 2013.

[4] J. M. Park, S. Josan, T. Jang, M. Merchant, R. Hurd, D. Mayer, L. Recht, and D. Spielman, "*Metabolic Changes in a Rat Glioma Model After Anti-Angiogenic Treatment Measured by MR Spectroscopic Imaging of Hyperpolarized [1-13C]Pyruvate*," presented at the Scientific Meeting of the ISMRM, Milan, Itraly.

[5] J. H. Ardenkjær-Larsen, A. M. Leach, N. Clarke, J. Urbahn, D. Anderson, and T. W. Skloss, "Dynamic nuclear polarization polarizer for sterile use intent," *NMR Biomed.*, vol. 24, no. 8, pp. 927–932, Mar. 2011.

[6] R. E. Hurd, Y.-F. Yen, A. Chen, and J. H. Ardenkjaer-Larsen, "Hyperpolarized 13C metabolic imaging using dissolution dynamic nuclear polarization," *J. Magn. Reson. Imaging*, vol. 36, no. 6, pp. 1314–1328, Nov. 2012.

[7] I. Park, R. Bok, T. Ozawa, J. J. Phillips, C. D. James, D. B. Vigneron, S. M. Ronen, and S. J. Nelson, "Detection of early response to temozolomide treatment in brain tumors using hyperpolarized 13C MR metabolic imaging," *J. Magn. Reson. Imaging*, vol. 33, no. 6, pp. 1284–1290, May 2011.

[8] J. M. Park, L. D. Recht, S. Josan, M. Merchant, T. Jang, Y. F. Yen, R. E. Hurd, D. M. Spielman, and D. Mayer, "Metabolic response of glioma to dichloroacetate measured in vivo by hyperpolarized 13C magnetic resonance spectroscopic imaging," *Neuro-Oncology*, vol. 15, no. 4, pp. 433–441, Mar. 2013.

[9] M. J. Albers, R. Bok, A. P. Chen, C. H. Cunningham, M. L. Zierhut, V. Y. Zhang, S. J. Kohler, J. Tropp, R. E. Hurd, Y.-F. Yen, S. J. Nelson, D. B. Vigneron, and J. Kurhanewicz, "Hyperpolarized 13C lactate, pyruvate, and alanine: noninvasive biomarkers for prostate cancer detection and grading.," *Cancer Research*, vol. 68, no. 20, pp. 8607–8615, Oct. 2008.

[10] M. M. Darpolor, Y.-F. Yen, M.-S. Chua, L. Xing, R. H. Clarke-Katzenberg, W. Shi, D. Mayer, S. Josan, R. E. Hurd, A. Pfefferbaum, L. Senadheera, S. So, L. V. Hofmann, G. M. Glazer, and D. M. Spielman, "In vivo MRSI of hyperpolarized [1-13C]pyruvate metabolism in rat hepatocellular carcinoma," *NMR Biomed.*, vol. 24, no. 5, pp. 506–513, Dec. 2010.

[11] M. M. Chaumeil, T. Ozawa, I. Park, K. Scott, C. D. James, S. J. Nelson, and S. M. Ronen, "Hyperpolarized 13C MR spectroscopic imaging can be used to monitor Everolimus treatment in vivo in an orthotopic rodent model of glioblastoma," *NeuroImage*, vol. 59, no. 1, pp. 193–201, Jan. 2012.

[12] S. E. Day, M. I. Kettunen, M. K. Cherukuri, J. B. Mitchell, M. J. Lizak, H. D. Morris, S. Matsumoto, A. P. Koretsky, and K. M. Brindle, "Detecting response of rat C6 glioma tumors to radiotherapy using hyperpolarized [1-13C]pyruvate and 13C magnetic resonance spectroscopic imaging," *Magnetic Resonance in Medicine*, vol. 65, no. 2, pp. 557–563, Nov. 2010.

[13] S. J. Nelson, J. Kurhanewicz, D. B. Vigneron, P. E. Z. Larson, A. L. Harzstark, M. Ferrone, M. van Criekinge, J. W. Chang, R. Bok, I. Park, G. Reed, L. Carvajal, E.

J. Small, P. Munster, V. K. Weinberg, J. H. Ardenkjaer-Larsen, A. P. Chen, R. E. Hurd, L. I. Odegardstuen, F. J. Robb, J. Tropp, and J. A. Murray, “Metabolic Imaging of Patients with Prostate Cancer Using Hyperpolarized [1-13C]Pyruvate,” *Sci Transl Med*, vol. 5, no. 198, pp. 198ra108–198ra108, Aug. 2013.

Appendix A: Inclusion/Exclusion Criteria Checklist

*All subject files must include supporting documentation to confirm subject eligibility. The method of confirmation can include, but is not limited to, laboratory test results, radiology test results, subject self-report, and medical record review.

Inclusion Criteria (From IRB approved protocol)	**Yes**	**No**	**Not Applicable**	**Supporting Documentation***
1. (Arm 2 patients with known CNS malignancy only) Patients with a known diagnosis of CNS malignancy, including metastases, with known enhancement on MR who are otherwise eligible to undergo MRI	☐	☐	☐	
2. At least 18 years old	☐	☐		
3. eGFR > 30 ml/min	☐	☐		
4. (Arm 2 patients with known CNS malignancy only) No allergy to gadolinium	☐	☐		
5. Ability to understand and willingness to sign a written informed consent document	☐	☐		
Exclusion Criteria (From IRB approved protocol)				
1. Refusal to have an IV placed for injection	☐	☐		
2. Acute major illness (e.g., unstable angina, etc.) or other condition that makes participation unsafe, per the investigator's judgement	☐	☐		
3. Total Bilirubin > 1.5 x ULN; AST/ALT > 2.5 x ULN; GGT > 2.5 x ULN	☐	☐		
4. Pregnant or breast-feeding	☐	☐	☐	
5. Cardiovascular risk, including: o Poorly controlled hypertension, defined as either systolic > 170 or diastolic > 110. o Congestive heart failure o Myocardial infarction within the past year o QT prolongation, defined as pretreatment QTc > 440 msec in males or > 460 msec in females	☐	☐		
6. (Arm 1 Healthy volunteers only) Undergoing active treatment for malignancy	☐	☐	☐	

IV. Statement of Eligibility

This subject is [☐ **eligible** / ☐ **ineligible**] for participation in the study.

Signature:	Date:
Printed Name:	