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TITLE: Simultaneous Multinuclear (Na+/H+) Metabolic MRI For Sodium-, pH-, and Oxygen-Sensitive Images in Human Brain Tumors

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14. ABSTRACT

Over the last reporting period we have (Aim 1) completed an interleaved ^{1}H-CEST-EPI/23Na-Radial FLASH imaging sequence and can collect both static and dynamic pH, O_2, and sodium-weighted MR information. We have enrolled 18 pre-operative patients (Aim 2) and obtained image-guided biopsies of areas thought to have high acidity, hypoxia, and sodium concentration with the goal of next correlating with tissue biology including NHE1 expression on IHC and RNA sequencing, as well as correlating with bioenergetics. Preliminary data shows a significant difference in NHE1 IHC expression in biopsy samples of high rNHE imaging contrast. Lastly (Aim 3), we have obtained pre- and post-immunotherapy pH, O_2, and sodium-weighted MR images in 13 patients with the goal of correlating with changes in patient outcome and tissue biology.

15. SUBJECT TERMS None listed.

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TABLE OF CONTENTS **Page**

1. Introduction

Glycolysis is often enhanced in cancers, *even in the presence of abundant oxygen* (i.e. the Warburg effect). This form of *aerobic glycolysis* results in a significant decrease in extracellular pH due to increased concentration of lactic acid and other factors. Maintaining pH regulation during excessive metabolism requires active transport of protons out of tumor cells. In brain cancer, this is done not only through lactate transport, but also through activity of sodium (Na^+)-proton (H^+) exchanger isoform-1 (NHE1). NHE1 is coupled with pH homeostasis in glioma cells and plays a role in in the progression of malignant gliomas as well as treatment resistance to both chemotherapies and immunotherapies, presumably because extracellular acidity has been linked to elevated mutagenesis and chromosomal rearrangements, elevated p53 and p21 expression, increased tumor invasion, formation of cancer stem cells, decreased immune function, and increased pro-angiogenic signaling. We hypothesize images of tumor acidity, oxygen consumption, and salinity (sodium concentration) can be performed in clinically realistic time frames through interleaving fast multinuclear Na^+/H^+ image acquisition. Such data will be useful for studying NHE1 expression and function in human brain tumors, including predicting immunotherapy treatment response. To accomplish this goal, we propose (Aim 1) constructing and testing a novel multinuclear Na^+-H^+ metabolic MRI sequence with sensitivity to Na^+ concentration, tissue pH, and O_2 utilization. Then (Aim 2) we will correlate Na^+-, pH-, and O_2-weighted MR image measurements with NHE1 IHC, bioenergetics, and gene expression using stereotactic image-guided biopsies from human brain tumors. Lastly, (Aim 3) we will quantify changes in Na^+-, pH-, and O_2-weighted MR images after neoadjuvant anti-PD-1 immunotherapy in recurrent GBM and explore associated changes in tumor biology.

2. Keywords

Glioblastoma; metabolic imaging; multinuclear MRI; sodium MRI; brain tumor; immunotherapy

3. Accomplishments

Aim 1: Construct and test a novel multinuclear Na+-H+ metabolic MRI sequence with sensitivity to Na+ concentration, pH, and O_2.

Major Task 0: IRB and HRPO Approval
Major Goals: IRB and HRPO Approval for Aim 1
Accomplishments: We accomplished this key objective within the first 3 months of last cycle. We continue to have IRB and HRPO approval through the current reporting period.

Major Task 1: Sequence Programming
Major Goals: Implement a prototype Na+/H+-CEST-SAGE-EPI sequence.
Accomplishments: As mentioned last report, the first year of this project we worked diligently with Siemens and collaborators to overcome software and firmware challenges associated with dynamically switching between excitation and reception of multiple nuclei within the same MRI pulse sequence. By the end of last report, we were happy to report that we overcame many of these challenges. To recap, at the end of the last reporting period we successfully acquired an interleaved, multinuclear spectroscopic free induction decay (FID) signal from both sodium and proton nuclei (or any X-nuclei, including ^{31}P). Next, we extended this basic spectroscopic sequence to include an interleaved multinuclear fast low angle shot gradient echo *imaging* sequence (^{1}H-FLASH/^{23}Na-FLASH) and demonstrated feasibility using a simple saltwater phantom. Lastly, we prototyped a single echo ^{1}H-CEST-EPI/^{23}Na-FLASH sequence, a critical step allowing pH and sodium-weighted image information simultaneously. However, by the end of the last reporting period we were not yet able to get this sequence to work correctly.

During the *current* reporting period, we finalized a working prototype of the ^{1}H-CEST-EPI/^{23}Na-FLASH sequence utilizing a radial-based acquisition trajectory for the ^{23}Na readout (**Fig. 1A-B**). We then created a multi-compartment phantom (**Fig. 1C**) consisting of 8 compartments with varying sodium concentration and pH (**Fig. 1D**). The resulting interleaved pH-sensitive amine CEST images (**Fig. 1E**) and sodium-weighted images (**Fig. 1G**) were of high quality, and when comparing the interleaved measurements to sequential (mononuclear) measurements there was high interclass correlation (ICC) for both amine CEST contrast (**Fig. 1F**) and relative sodium signal (**Fig. 1H**).

It is important to note that the interleaved multinuclear MR sequences will only run on multinuclear coils with both transmit and receive (excitation and acquisition) capabilities. As such, our sequence will only run correctly on our extremity volume coil and *will unfortunately not run correctly on our multinuclear head coil*. Our head coil is a single channel transmit/receive for ^{23}Na, but is a multichannel *receive only* for the proton nuclei (relying on the MR system body coil to transmit RF energy for ^{1}H excitation).

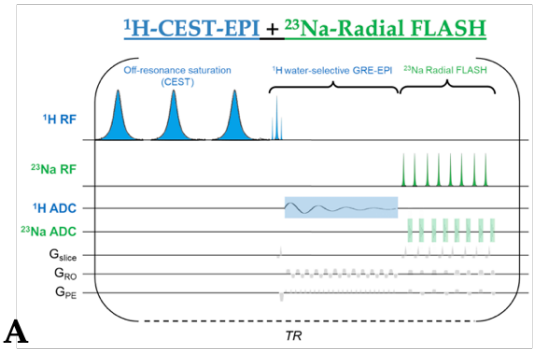
Therefore, to test the operation of our sequence, we designed a lower leg exercise paradigm and altered our interleaved sequence to obtain *dynamic* pH-, sodium-, and oxygen-sensitive measurements of the calf muscle during recovery (**Fig. 1I**). By dynamically toggling between *z*-spectrum offset frequencies during acquisition (**Fig. 1J**) and performing a sliding temporal window while applying the Golden-angle RAdial Sparse Parallel (GRASP) technique for reconstructing the radial ^{23}Na images, we successfully created interleaved, multinuclear images over time (**Fig. 1K**). The average ^{23}Na, BOLD T2* signal, and pH-sensitive CEST signal over time showed distinctly different time constants for these different processes. Together, the phantom data combined with this dynamic *in vivo* experiment provides evidence of successful completion of this task, with the caveat that it cannot be run on the brain due to the type of coils available currently at our center.

Major Task 2: Phantom Testing
Major Goals: Repeated testing on a custom phantom to ensure stability and accuracy of the new MRI pulse sequence.
Accomplishments: We have constructed a saltwater and pH-sensitive multi-compartment phantom for use in the current project as outlined in **Fig. 1C-D**. We have performed extensive testing on this sequence (**Fig. 1E-H**) and verify it is fully operational. This data was presented at the International Society for Magnetic Resonance in Medicine (ISMRM) 2023 Annual Meeting in Toronto.

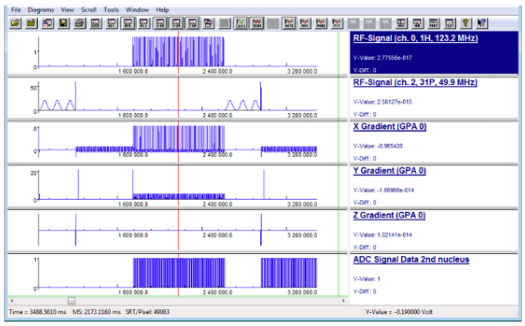
Major Task 3: Human Testing
Major Goals: Test the stability and repeatability of the MRI pulse sequence in 20 healthy volunteers.

Accomplishments: As mentioned previously, we are *not* able to acquire *in vivo* data with our interleaved multinuclear sequence in the brains of volunteers because the head coil we have is not capable of transmitting and receiving both proton and sodium nuclei. Instead, to demonstrate the operation of this sequence, we utilized our transmit/receive volume extremity coil and are conducting a lower leg exercise paradigm and altered our interleaved sequence to obtain *dynamic* pH-, sodium-, and oxygen-sensitive measurements of the calf muscle during recovery (**Fig. 1I-L**). To date, we have run 2 healthy volunteers, but have IRB approval to finish all 20 volunteers.

Interleaved ^{1}H-CEST-EPI/^{23}Na-Radial FLASH Pulse Sequence

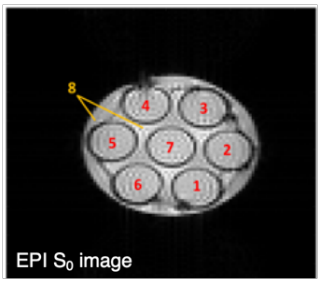


A



B

Multi-Compartment Phantom



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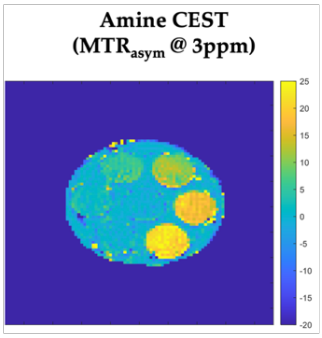

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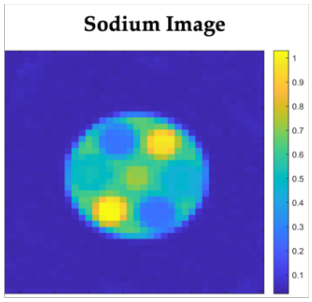
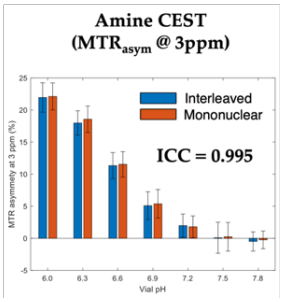


G

Vial	pH	NaCl (mM)
1	6.0	25
2	6.3	50
3	6.6	100
4	6.9	25
5	7.2	50
6	7.5	100
7	7.8	75
8	-	75

D



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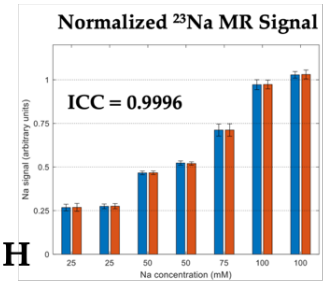
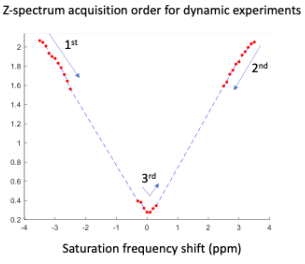


H

Dynamic ^{1}H-CEST-EPI + ^{23}Na-Radial FLASH

I



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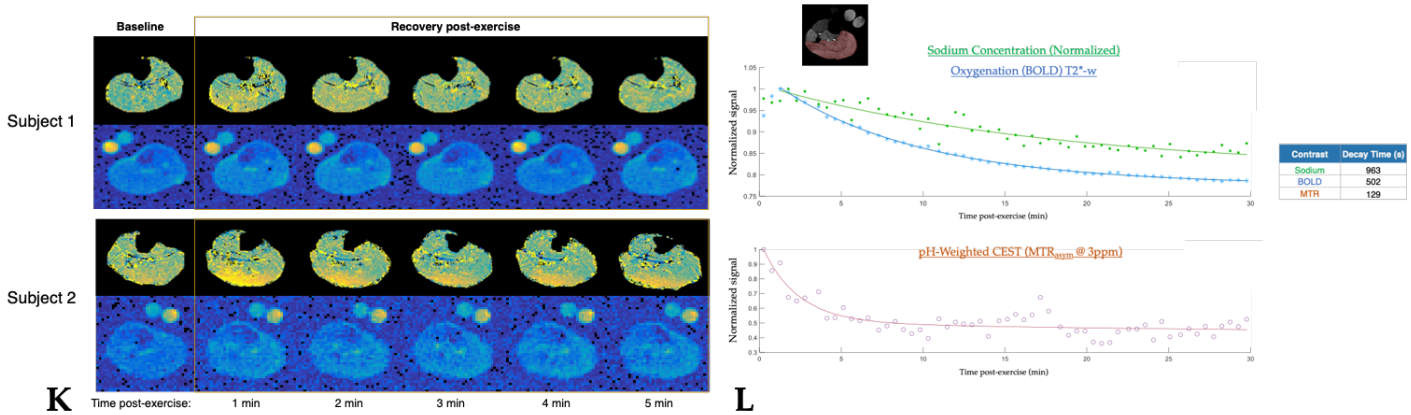

K L

Fig. 1. A) Interleaved multinuclear ^{1}H-CEST-EPI/^{23}Na-Radial FLASH pulse sequence diagram and **B)** same diagram shown in Siemens IDEA development software environment. **C)** ^{1}H-EPI S_0 images (T2*-weighted) showing a multicompartmental phantom. **D)** Table showing the pH and sodium concentrations in each of the compartments in the phantom. **E)** Resulting pH-weighted amine CEST images (MTR_{asym} at 3ppm) showing adequate pH sensitivity. **F)** Comparison between interleaved and separately acquired pH-sensitive amine CEST-EPI measurements confirming good fidelity of measurements as indicated by high interclass correlation (ICC = 0.995). **G)** Sodium-weighted image of the same phantom and **H)** relative sodium MR signal intensity using interleaved versus sequential acquisition, again confirming high fidelity as indicated by the high ICC = 0.9996. **I)** To test this new sequence *in vivo*, we designed an exercise recovery experiment using *dynamic* ^{1}H-CEST-EPI/^{23}Na-Radial FLASH imaging of the calf muscle after rigorous exercise. To do this, we toggled between *z*-spectral points within ^{1}H-CEST-EPI acquisition and used a sliding window and GRASP reconsctruction for the sodium images. **K)** Results from first two healthy control subjects with pH-sensitive CEST-EPI images on the top and sodium-weighted images on the bottom over time. **L)** Average plots from the soleus muscle in one patient shows differing responses for oxygen, pH, and sodium after exercise.

Aim 2: Correlate Na^+-, pH-, and O_2-weighted MR image measurements with IHC, bioenergetics, and gene expression using stereotactic image-guided biopsies from human brain tumors.

Major Task 0: IRB and HRPO Approval

Major Goals: IRB Approval for Aim 2.

Accomplishments: Within the first 3 months we accomplished this key objective and established IRB and HRPO approval. This task is complete.

Major Task 1: Surgical Patient Recruitment

Major Goals: Start recruitment of newly diagnosed or recurrent gliomas.

Accomplishments: At the end of the *previous* progress report, we enrolled 6 patients to this study. To date, we have recruited **18 patients** with new or recurrent gliomas to the study. This is back in line with the expected enrollment of 1-2 patients per month. Prior recruitment issues were due to COVID-19 concerns, difficulty scheduling patients on campus during COVID-19 shut-downs, and technical issues related to the coil and MR scanner itself. We have overcome these issues and have since recruited 18 of the target 20 patients.

Major Task 2: Na+/H+ Pre-Surgical MRI

Major Goals: MRI exam including exploratory Na+/H+ sequences, the international standardized brain tumor imaging protocol (BTIP), diffusion and perfusion imaging.

Accomplishments: We have successfully acquired multinuclear imaging data in all 18 patients we have enrolled. The exam is about 90 minutes and covers the standard anatomic sequences within BTIP along with sodium MRI acquired at the very end, after contrast administration. We will continue to acquire pre-surgical multinuclear MRI scans in the remaining 2 patients over the next year.

Major Task 3: Surgery & Correlation with Tissue

Major Goals: Surgery and correlation with tissue.

Accomplishments: We have successfully collected multiple biopsies in each of the 18 enrolled patients (Subtask 1). Tissue is currently being banked and bioenergetics/seahorse (Subtask 2), NHE1 RNA seq (Subtask 3), and NHE1 IHC staining (Subtask 4) are currently being performed. To date, we have processed NHE1 IHC (Subtask 4) in 28 biopsy samples from 8 of the first patients enrolled (**Fig. 2**). Results clearly show that biopsies obtained in rNHE images (composite images created by combining the multinuclear images) of low contrast correspond with a lower proportion of cells with positive NHE1 IHC stain (**Fig. 2A**) compared with areas of high rNHE contrast (**Fig. 2B**). Independent scoring of NHE1 nuclear staining density (from 0 to +3) confirmed that high rNHE *imaging* contrast was higher in biopsy samples showing high NHE1 expression (**Fig. 2C**; *P=0.0003*). Receiver-operator characteristic (ROC) curves confirmed that rNHE *imaging* measurements could reliably identify biopsy tissue with relatively high NHE1 expression (*ROC AUC = 0.918, P=0.0002*), with an rNHE imaging measurement >0.75 resulting in approximately an 80% sensitivity and specificity for identifying tissue with high NHE1 expression (**Fig. 2D**). Comparatively, relative sodium concentration on sodium-weighted images (**Fig. 2E**; *P=0.1734*) and pH-weighted CEST contrast (**Fig. 2F**; *P=0.2277*) were no different between tumor tissue with high or low NHE1 expression.

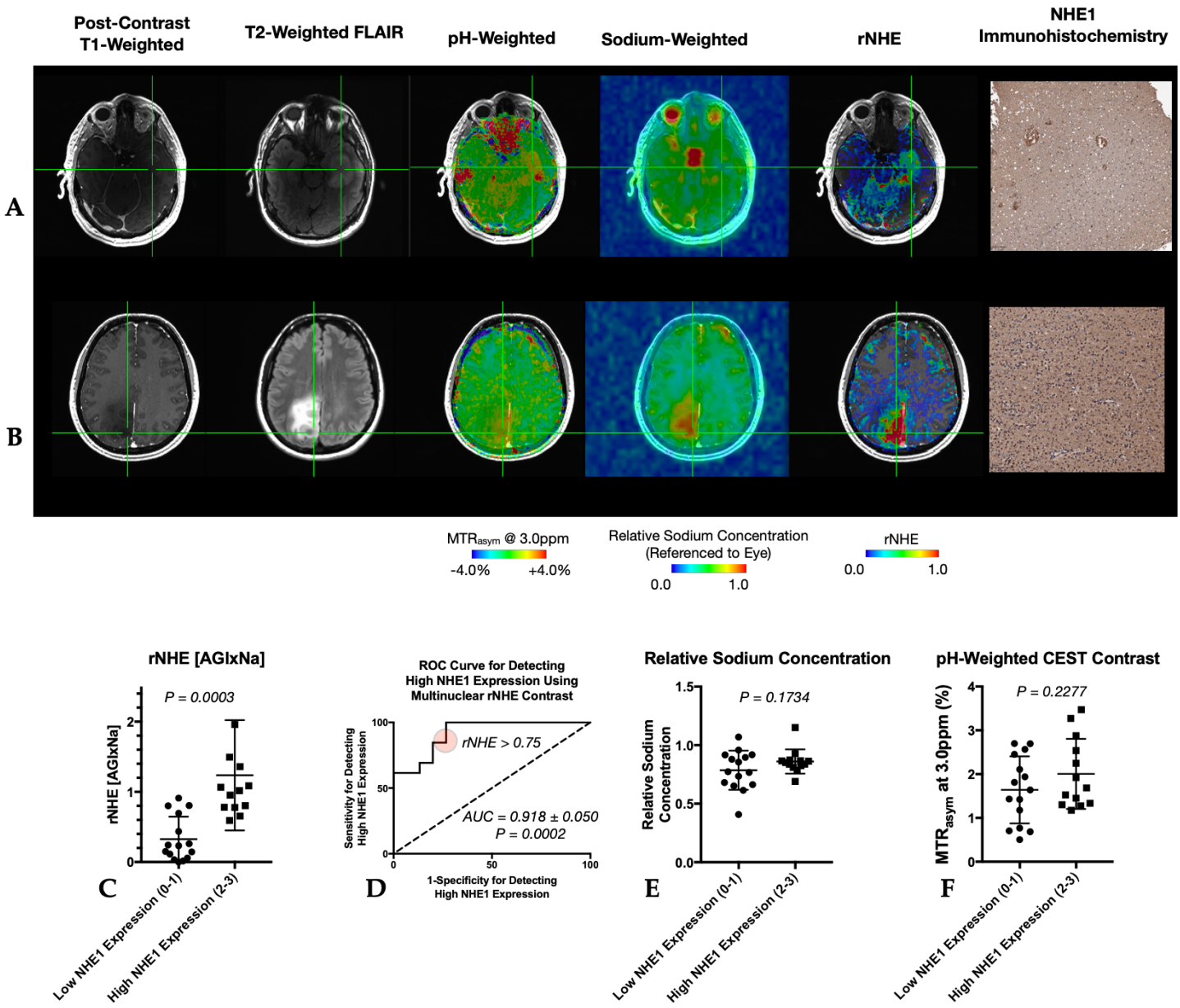

Fig. 2. A) Biopsy patient with relatively low rNHE contrast and **B)** patient with relatively high rNHE contrast (Left to right) Post-contrast T1-weighted images, T2-weighted FLAIR images, pH-weighted amine CEST-EPI images, sodium-weighted images, relative sodium-hydrogen exchanger (rNHE) images based on a combination of sodium-, pH-, O_2-, diffusion-, and perfusion-weighted images, and (far right) images show resulting NHE1 immunohistochemistry images of biopsied areas represented by crosshairs. **C)** Comparison of rNHE *imaging* contrast measurements within biopsy regions in samples determined to have low NHE1 expression (left, score of 0 to +1) and high NHE1 expression (right, score +2 to +3). **D)** ROC curve for detecting high NHE1 expression using rNHE imaging contrast. **E)** Comparison of relative sodium concentration and **F)** pH-weighted CEST measurements in biopsy regions with low and high NHE1 expression.

Aim 3: Quantify changes in Na+-, pH-, and O2-weighted MR images after neoadjuvant anti-PD-1 immunotherapy in recurrent GBM and explore associated changes in tumor biology.

Major Task 0: IRB and HRPO Approval

Major Goals: IRB Approval for Aim 3.

Accomplishments: Within the first 3 months we accomplished this key objective and established IRB and HRPO approval. This task is complete.

Major Task 1: Recurrent Patient Recruitment

Major Goals: Start recruitment of recurrent glioblastoma patients being treated anti-PD-1 immunotherapy.

Accomplishments: At the end of the *previous* reporting period, we enrolled only 5 patients for the same reasons outlined for Aim 2 (COVID-19 lock down and technical issues with the coil and scanner). Over this latest reporting period, we have successfully recruited **13 patients** with recurrent glioblastoma undergoing immunotherapy. We are on target to finish enrollment during this next study period.

Major Task 2: Pre/Post Treatment Scanning

Major Goals: Major goals for this task include acquiring a pre-treatment multinuclear MRI exam (Subtask 1), treatment with anti-PDL1 immunotherapy (Keytruda) (Subtask 2), and acquiring a post-treatment multinuclear MRI exam (Subtask 3) prior to surgery.

Achievements: We have successfully acquired pre-treatment multinuclear MRI exams in **13 patients** enrolled in Aim 3 at the end of this reporting period. While we are currently analyzing data and correlating it with outcomes, we have noticed a strong linear correlation between the size of enhancing tumor and average rNHE imaging contrast (**Fig. 3**).

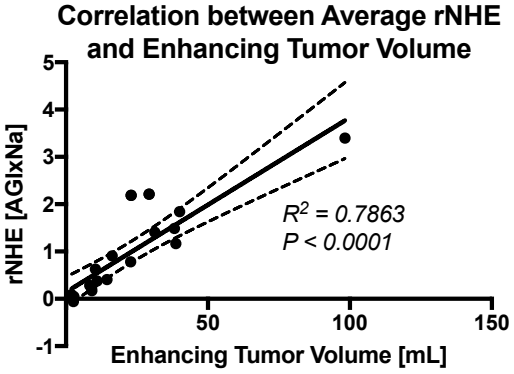

Fig. 3. Correlation between average rNHE imaging contrast on multinuclear images and contrast enhancing tumor volume.

Major Task 3: Correlation with Tissue

Major Goals: Surgery and correlation with tissue.

Accomplishments: We have successfully collected tissue from patients enrolled in this aim. All tissue is currently banked, and analysis is ongoing in collaboration with Dr. Rob Prins. Results will be evaluated later this year.

Major Task 4: Correlation with Outcomes

Major Goals: Correlate rNHE response with progression-free (PFS) and overall survival (OS).

Accomplishments: We are currently examining both PFS and OS in the 13 patients enrolled in this aim.

Additional Findings

One of our secondary goals for this project was to determine the association between these multiparametric and multinuclear measurements to see of some of the information is redundant. Results from the data so far has shown both relative sodium concentration and the apparent diffusion coefficient of water (ADC) were significantly higher in areas of necrosis compared to non-enhancing tumor (NET) ($P = 0.003$ and $P = 0.008$, respectively) and contrast-enhancing tumor (CET) ($P = 0.02$ and $P = 0.02$). Sodium concentration was higher in CET compared to NET ($P = 0.04$). Sodium and ADC were higher in treated compared to treatment-naïve gliomas within NET ($P = 0.006$ and $P = 0.01$, respectively), and ADC was elevated in CET ($P = 0.03$). Median ADC and sodium concentration were positively correlated across patients in NET ($r = 0.77$, $P < 0.0001$) and CET ($r = 0.84$, $P < 0.0001$), but not in areas of necrosis ($r = 0.45$, $P = 0.12$). Median nrCBV and sodium concentration were negatively correlated across patients in areas of NET ($r=-0.63$, $P = 0.003$). Similar associations were observed when examining voxel-wise correlations within VOIs. These data were presented at the *Society of Neuro Oncology (SNO) Annual Meeting* in November 2022 and later published in (including the journal cover):

Cho NS, Sanvito F, Thakuria S, Wang C, Hagiwara A, Nagaraj R, Oshima S, Lopez Kolkovsky AL, Lu J, Raymond C, Liau LM, Everson RG, Patel KS, Kim W, Yang I, Bergsneider M, Nghiemphu PL, Lai A, Nathanson DA, Cloughesy TF, Ellingson BM. Multi-nuclear sodium, diffusion, and perfusion MRI in human gliomas. J Neurooncol. 2023 Jun;163(2):417-427. doi: 10.1007/s11060-023-04363-x. Epub 2023 Jun 9. PMID: 37294422; PMCID: PMC10322966.

Opportunities for Training and Professional Development

This project has led to a few training and professional development opportunities. Nicholas Cho, an MD/PhD graduate student in my laboratory, and Dr. Francesco Sanvito, a postdoctoral fellow and neuroradiologist, have been working together to spearhead the clinical components of this study. This project has allowed them training in image acquisition and analyses, and they have learned numerous skills related to image-guided biopsy from Aim 2. Nicholas and Francesco have attended several surgeries and have obtained precise targets in the O.R. as neurosurgeons take out the tissue for subsequent analyses. Additionally, Nicholas has presented a poster at the *Society of Neuro Oncology (SNO) Annual Meeting* last fall in Tampa.

Dissemination of Results

Some of the results mentioned above were presented at the American Society of Neuroradiology (ASNR) Annual Meeting in Chicago April 29-May3, 2023, as well as the joint Society of Neuro Oncology (SNO) and American Society of Clinical Oncology (ASCO) Meeting on CNS Clinical Trials in San Francisco, August 10-12, 2023. At these meetings, we described our approach as a way of understanding changes in the microenvironment after immunotherapy and as an example of imaging technology on the horizon.

An abstract related to some of the data outlined above was recently presented as a poster at the 2022 SNO Annual Meeting.

Plan to Accomplish Remaining Goals During Next Reporting Period

We have completed the complex multinuclear MRI pulse sequence outlined for **Aim 1**; however, we are not able to acquire brain MRI data due to the type of coils available and limitations on the pulse sequence. Therefore, we will be acquiring data using the dynamic exercise recovery paradigm outlined in **Fig. 1** to show proof-of-principle.

We are prioritizing and rapidly increasing patient enrollment for **Aims 2-3** over the next reporting period to reach our proposed target enrollment goals. Additionally, we will be finalizing tissue processing for the remaining cases over the next reporting period.

4. Impact

Impact on the Development of Principle Disciplines of the Project

During a series of scientific meetings, we described strategies aimed at unraveling the intricate interplay between sodium levels, acidity, and hypoxia within the tumor microenvironment. Our goal was to shed light on how these factors could serve as predictive indicators for patient responsiveness to immunotherapies, and whether fluctuations in tumor characteristics, potentially stemming from inflammatory processes, influenced the tumor's microenvironment. Our initial findings have ignited new conversations surrounding the potential utility of our tool, alongside groundbreaking composite biomarkers like spatial transcriptomics, to deepen our comprehension of how the tumor microenvironment's traits may influence the mechanisms of therapeutic action and, consequently, treatment effectiveness.

Impact on Other Disciplines

Some of the technology developed in this application has had impact on other disciplines. For example, **Fig. 1** shows that this technology can be used to quantify *dynamic* effects on the tissue microenvironment. Additionally, we show how this same technology can be used to quantify and visualize muscle recovery from intensive exercise, suggesting this same technology may be useful for musculoskeletal applications or even military/fitness science.

Additionally, we have also been collaborating with Siemens engineers about integrating our interleaved multinuclear sequences for ultra-high field (UHF) 7T MR scanners.

Impact on Technology Transfer

We are working in collaboration with Siemens on this project and therefore anticipate our technology to be useful on their multinuclear MRI systems including UHF systems.

Impact on Society Beyond Science and Technology

Nothing to Report

5. Changes/Problems

Changes in Approach and Reasons for Change

We have not changed our approach during the reporting period.

Actual or Anticipated Problems or Delays and Actions or Plans to Resolve Them

Over the last reporting period we have not had any actual or anticipating problems or delays. As mentioned in the previous (1st year) technical report, we had some major problems that significantly delayed enrollment including (1) issues associated with technically acquiring two different nuclei in the same pulse sequence, a critical step in building a multinuclear MRI pulse sequence; and (2) technical issues with the multinuclear MRI head coil and MR system itself. However, these issues were resolved early during the last (2nd year) period and we have increased our enrollment to expected levels. We do not anticipate any issues until the end of this project.

Changes that had a Significant Impact on Expenditures

Nothing to report.

Significant Changes in Use or Care of Human Subjects

Nothing to report.

The IRB for Aim 1 (IRB#21-000234) is currently approved until 1/23/2024.
The IRB for Aims 2-3 (IRB#21-000514) is currently approved until 3/23/2024

6. Products

Nothing to report.

7. Individuals that have worked on the project

Name:	Benjamin M. Ellingson, Ph.D.
Project Role:	Principal Investigator
Researcher Identifier (ORCID ID):	0000-0002-2764-6640
Nearest Person Month Worked:	2.4
Contribution to the Project:	Dr. Ellingson provides oversight and expertise for all aspects of the current study. He is involved in pulse sequence programming tasks, enrolling and scanning patients, performing image analysis, and identifying biopsy targets.
Funding Support:	UCLA Department of Radiology Support; NIH/NCI Brain Tumor SPORE grant; NIH/NCI R01 grant; NIH/NINDS R01 grant

Name:	Jingwen Yao, Ph.D.
Project Role:	Graduate Student
Researcher Identifier (ORCID ID):	0000-0002-8828-1841
Nearest Person Month Worked:	1.0
Contribution to the Project:	Dr. Yao was involved in early pulse sequence programming and image analysis tasks (Aim 1) before she left the University for fellowship in July 2021.
Funding Support:	UCLA Department of Radiology Support; NIH/NCI Brain Tumor SPORE grant

Name:	Mark Bydder, Ph.D.
Project Role:	Staff Scientist
Researcher Identifier (ORCID ID):	0000-0001-9210-0225
Nearest Person Month Worked:	1.0
Contribution to the Project:	Dr. Bydder was involved in early pulse sequence programming tasks (Aim 1) before he left the University in late 2021.
Funding Support:	UCLA Department of Radiology support

Name:	Chencai Wang, Ph.D.
Project Role:	Staff Scientist/Programmer
Researcher Identifier (ORCID ID):	0000-0003-0501-3073
Nearest Person Month Worked:	2.4
Contribution to the Project:	Dr. Wang has performed work related to designing and implementing the multinuclear MRI sequence (Aim 1).
Funding Support:	UCLA Department of Radiology Support; NIH/NCI Brain Tumor SPORE grant; NIH/NCI R01

Name:	Nicholas Cho, B.S.
Project Role:	Graduate Student
Researcher Identifier (ORCID ID):	0000-0002-8686-0575
Nearest Person Month Worked:	2.4
Contribution to the Project:	Mr. Cho is involved in image acquisition, post-processing, and analysis for Aims 2-3.

Funding Support:	UCLA Department of Radiology Support; NIH/NCI Brain Tumor SPORE grant; UCLA NIH T32 Medical Scientist Training Program

Name:	Francesco Sanvito, M.D.
Project Role:	Visiting Medical Scholar
Researcher Identifier (ORCID ID):	0000-0002-9152-9684
Nearest Person Month Worked:	1.0
Contribution to the Project:	Dr. Sanvito has assisted in image acquisition, post-processing, and analysis for Aims 2-3.
Funding Support:	UCLA Department of Radiology Support

Name:	Catalina Raymond, M.S.
Project Role:	Lead Programmer
Researcher Identifier (ORCID ID):	0000-0003-2757-439X
Nearest Person Month Worked:	1.0
Contribution to the Project:	Ms. Raymond is responsible for data processing, QC/QA, storage, and analysis for all aspects of the project.
Funding Support:	UCLA Department of Radiology Support

Name:	David Nathanson, Ph.D.
Project Role:	Co-Investigator
Researcher Identifier (ORCID ID):	0000-0002-4919-9159
Nearest Person Month Worked:	1.0
Contribution to the Project:	Dr. Nathanson is responsible for tissue processing for Aim 2.
Funding Support:	NIH/NCI Brain Tumor SPORE; Multiple NIH/NCI R01s

Name:	Linda Liau, M.D., Ph.D.
Project Role:	Co-Investigator
Researcher Identifier (ORCID ID):	0000-0002-4053-0052
Nearest Person Month Worked:	0.5
Contribution to the Project:	Dr. Liau is responsible for patient recruitment and obtaining image-guided biopsies for Aim 2.
Funding Support:	NIH/NCI Brain Tumor SPORE; Multiple NIH/NCI R01s

Name:	Robert Prins
Project Role:	Co-Investigator
Researcher Identifier (ORCID ID):	0000-0002-6282-6583
Nearest Person Month Worked:	0.5
Contribution to the Project:	Dr. Prins is responsible for immunology tissue processing for Aim 3.
Funding Support:	NIH/NCI Brain Tumor SPORE; Multiple NIH/NCI R01s

Name:	Harley Kornblum
Project Role:	Co-Investigator
Researcher Identifier (ORCID ID):	0000-0002-3779-4540
Nearest Person Month Worked:	0.5
Contribution to the Project:	Dr. Kornblum is responsible for single cell and stem cell analyses for Aim 2.
Funding Support:	NIH/NCI Brain Tumor SPORE

Changes in the Active Other Support for the PIs or Senior/Key Personnel Since Last Reporting Period:

Benjamin M. Ellingson, Ph.D.

- **National Institutes of Health (NIH) and National Cancer Institute (NCI) R01 CA270027-01:**
  Role of decorin and diffusion MRI in anti-VEGF efficacy for recurrent glioblastoma:
  This new project will establish the mechanistic links between decorin (DCN) expression, diffusion MRI, and anti- VEGF treatment efficacy by first (Aim 1) performing a deep exploration into the association between diffusion MR phenotypes and DCN expression in human GBM using image-guided biopsies and examining DCN protein and gene expression, as well as the relationship with genotypes using whole exome analysis, genetic subtypes using bulk RNA sequencing, cellular states using single-cell RNA sequencing, and blood plasma levels of circulating DCN. Concurrently, we will (Aim 2) establish the causal links between DCN expression, diffusion MRI measurements, and anti-VEGF treatment in GBM by conducting a genetically modified patient-derived orthotopic xenograft (PDX) preclinical trial through editing a series of patient-derived cell lines to silence of overexpress DCN within PDX models using a tetracycline-controlled gene expression system, then perform diffusion MRI and treat with anti-VEGF therapy.

- **National Institutes of Health (NIH) R01CA279984:**
  Quantitative molecular MR-PET imaging of glycolysis in glioblastoma:
  The current study will investigate the central hypotheses that: (Aim 1) fast pH- and O2- weighted amine CEST spin-and-gradient-echo echoplanar imaging (CEST-SAGE-EPI) can be significantly improved by increasing speed, coverage, image quality, and accuracy using simultaneous multi-slice (SMS) acceleration, deeplearning,physics-based EPI artifact reduction techniques, and implementation of more quantitative CEST approaches; (Aim 2) biopsied tumor tissue undergoing high levels of glycolysis via metabolomic analyses, RNA expression, protein expression, and bioenergetics analyses can be reliably detected and correlates strongly with a "glycolytic index" created by combining 18F-FDG PET and amine CEST-SAGE- EPI; and (Aim 3) changes in this "glycolytic index" can be detected by perturbing glucose metabolism using a brain penetrant EGFR inhibitor specifically designed for GBM and correlate with pharmacologic alterations and alterations in glycolytic signaling in patients with IDH wild-type, EGFR amplified GBM.

- **DOD-CDMRP HT94252310899 (pending award processing at UCLA):**
  Targeting the EGRF Signaling Network in Glioblastoma:
  This project leverages an early phase clinical trial of ERAS-801, combined with our innovative preclinical GBM models, to concurrently (1) evaluate a non-invasive, metabolic imaging biomarker of treatment response; (2) study the biological changes that occur after acquired resistance (failure) of ERAS-801; and (3) test whether a combination treatment with ERAS-801 and a drug that inhibits of the anti-apoptotic protein BCL-xL enhance intrinsic GBM cell death.

David Nathanson, Ph.D.

- **National Brain Tumor Society 20193982:**
  Therapeutic Approaches that Target Apoptotic Blocks in GBM:
  Our goal is to determine the molecular mechanisms by which GBM tumors are resistant to intrinsic apoptosis. Moreover, we will explore novel drug combinations to target the intrinsic apoptotic machinery for enhanced GBM tumor cell death.

- **National Institutes of Health (NIH) and National Cancer Institute (NCI) R01CA270027:**
  Role of decorin and diffusion MRI in anti-VEGF efficacy for recurrent glioblastoma:
  Extensive preliminary data (>7 trials in >400 patients) suggests diffusion MRI characteristics are a strong, independent predictor of anti-VEGF therapeutic efficacy in recurrent GBM, with patients exhibiting a significant survival benefit if they present with a high apparent diffusion coefficient (ADC) within contrast enhancing tumor. Data also suggests these diffusion MR signatures may result from an elevated expression of decorin (DCN), a glycoprotein with a variety of functions. We hypothesize that the survival advantage and imaging signatures arise from the multifaceted functions of DCN, which include anti-angiogenic characteristics and softening of the extracellular matrix, which we theorize would result in increased effectiveness of anti-VEGF therapies and an increase in ADC. As such, the current study will explore the causal, mechanistic links between DCN expression, diffusion MRI, and anti-VEGF treatment efficacy.

- **NIH / University of alabama Birmingham R01CA258248:**
  Credentialing Next-Generation Human Glioma Models for Precision Therapeutics:
  This project seeks develop novel glioma preclinical models for the evaluation of targeted therapies against the epidermal growth factor receptor.

- **Boundless Bio, Inc 20224598:**
  Role of ecDNA in Glioblastoma multiform Preclinical Models:
  This collaboration seeks to investigate the presence and structure of ecDNA in GBM patient- derived xenograft (PDX) models, with particular focus on EGFR, CDK4/6, and MDM2 amplified cancers. The collaboration further seeks to evaluate the impact of Boundless Bio ecDNA-directed therapeutics (ecDTx) on ecDNA and their anti-tumor effects in a representative subset of orthotopic PDX models.

- **David Geffen School of Medicine at UCLA:**
  Neuro-Oncology Program:
  Support Neuro-Oncology program

- **Katmai Pharmaceuticals, Inc:**
  Efficacy and Pharmacodynamic evaluation of ERAS-201 and Tagrisso an Intracranial NSCLC PDX:
  To evaluate and compare the efficacy of Tagrisso or ERAS-801 in an intracranial PDX model of EGFR exon 19 mutant NSCLC, and to evaluate whether FDG PET can serve as a rapid, predictive biomarker of response to inhibition of EGFR signaling.

- **Katmai Pharmaceuticals, Inc:**
  Expansion Cohort to Evaluate the Impact of Specific Tumor Genotypes on response to ERAS-801:
  Preliminary data indicate that PTEN and/or NF1 alterations may impact the response of GBMs harboring EGFR polysomy alterations. This study aims to validate these findings using addition GBM PDOX models with/without alterations in PTEN and NF1. In addition, this study will further validate the potential for FDG PET to serve as an early predictive biomarker for response to ERAS-801.

- **Asteroid Theraputics, Inc:**
  Pre-clinical evaluation of novel modulators of lipid metabolism to assess efficacy across patient-derived GBM cells and identification of potential predictive biomarkers of response:
  The goal of this study is (1) to determine whether targeting SREBP and/or LXR – using 5 novel drug candidates developed by Asteroid Tx – has functional activity across molecularly heterogeneous patient

derived GBM gliomaspheres, and (2) to evaluate whether the expected efficacy studies proposed in Study 1 are translated in intracranial orthoptoic xenograft bearing molecularly characterized, patient-derived orthotopic xenografts.

- **National Institutes of Health (NIH)h and National Institute of Neurological Disorders and Stroke (NINDS) R01NS126849:**
  Programming multi-pronged immune response to glioblastoma with combinatorial CAR-T cell therapy:
  We aim to develop effective immunotherapy for glioblastoma multiforme (GBM) by deploying IL-13Rα2/TGF-β bispecific chimeric antigen receptor (CAR)-T cells that can both (a) directly target GBM through IL-13Rα2 surface-antigen recognition and (b) modify the tumor microenvironment (TME) by converting TGF-β from an immunosuppressant into a stimulant of engineered CAR-T cells. We will further evaluate the combination of IL- 13Rα2/TGF-β bispecific CAR-T cell therapy with PD-1 blockade and dendritic cell (DC) vaccination—two immunotherapy strategies that have shown synergistic potential by promoting T-cell activation and effector function. This work will systematically evaluate combination therapies in both syngeneic GBM tumor models and patient-derived GBM tumors, in order to identify the most effective treatment regimen and generate comprehensive preclinical data in support of a phase-1 clinical trial for GBM.

- **National Institutes of Health (NIH) R01CA279984:**
  Quantitative molecular MR-PET imaging of glycolysis in glioblastoma:
  The current study will investigate the central hypotheses that: (Aim 1) fast pH- and O2- weighted amine CEST spin-and-gradient-echo echoplanar imaging (CEST-SAGE-EPI) can be significantly improved by increasing speed, coverage, image quality, and accuracy using simultaneous multi-slice (SMS) acceleration, deeplearning,physics-based EPI artifact reduction techniques, and implementation of more quantitative CEST approaches; (Aim 2) biopsied tumor tissue undergoing high levels of glycolysis via metabolomic analyses, RNA expression, protein expression, and bioenergetics analyses can be reliably detected and correlates strongly with a "glycolytic index" created by combining 18F-FDG PET and amine CEST-SAGE- EPI; and (Aim 3) changes in this "glycolytic index" can be detected by perturbing glucose metabolism using a brain penetrant EGFR inhibitor specifically designed for GBM and correlate with pharmacologic alterations and alterations in glycolytic signaling in patients with IDH wild-type, EGFR amplified GBM.

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  Targeting the EGRF Signaling Network in Glioblastoma:
  This project leverages an early phase clinical trial of ERAS-801, combined with our innovative preclinical GBM models, to concurrently (1) evaluate a non-invasive, metabolic imaging biomarker of treatment response; (2) study the biological changes that occur after acquired resistance (failure) of ERAS-801; and (3) test whether a combination treatment with ERAS-801 and a drug that inhibits of the anti-apoptotic protein BCL-xL enhance intrinsic GBM cell death.

- **National Institutes of Health (NIH) and National Cancer Institute (NCI) R01CA270027: Role of decorin and diffusion MRI in anti-VEGF efficacy for recurrent glioblastoma:**
  Extensive preliminary data (>7 trials in >400 patients) suggests diffusion MRI characteristics are a strong, independent predictor of anti-VEGF therapeutic efficacy in recurrent GBM, with patients exhibiting a significant survival benefit if they present with a high apparent diffusion coefficient (ADC) within contrast enhancing tumor. Data also suggests these diffusion MR signatures may result from an elevated expression of decorin (DCN), a glycoprotein with a variety of functions. We hypothesize that the survival advantage and imaging signatures arise from the multifaceted functions of DCN, which include anti-angiogenic characteristics and softening of the extracellular matrix, which we theorize would result in increased effectiveness of anti-VEGF therapies and an increase in ADC. As such, the current study will explore the causal, mechanistic links between DCN expression, diffusion MRI, and anti-VEGF treatment efficacy.

- **National Institutes of Health (NIH) and National Cancer Institute (NCI) R01CA222695: Identification and Cloning of Neoantigen-specific T Cells for GBM Immunotherapy:**
  These studies span the continuum of translational research in brain tumor immunotherapy and will likely provide informative new insights for the development of new, rational immune-based strategies for brain tumor patients.

Robert Prins, Ph.D.

- **National Institutes of Health (NIH) and National Institute of Neurological Diseases and Stroke (NINDS) R01NS126849:**
  Programming Multi-pronged Immune Response to Glioblastoma with IL-13Ra2/TGF-ß CAR-T Cell Therapy:
  The goals are to evaluate the ability of IL-13Rα2/TGF-β CAR-T cells to directly eliminate IL-13Rα2+ GBM, as well as to induce endogenous immunity against IL-13Rα2– GBM, in both patient-derived orthotopic xenograft models and immunocompetent mouse models. Rigorously evaluate the safety and toxicity profile of IL-13Rα2/TGF-β CAR-T cell therapy and generate comprehensive preclinical data in support of a phase-1 clinical trial for the treatment of patients with GBM.

- **DOD/CDMRP HT94252310616:**
  Alternative Splice Variant-Derived Neoantigen-Targeted Immunotherapy in Diffuse Hemispheric glioma, H3 G34-Mutant:
  The goal is to develop and implement new methodologies pertaining to targeted immunotherapy in brain tumor, and my specific role is help to understand how immunotherapy modifies the tumor immune microenvironment

- **National Institutes of Health (NIH) and National Cancer Institute (NCI) R01CA267726:**
  Neoadjuvant Checkpoint blockade for recurrent Glioblastoma:
  The goals of this project are to test how combined neoadjuvant immunotherapy alters the systemic and local tumor microenvironment in recurrent glioblastoma patients.

- **The Glioblastoma Research Organization/Children's Hospital Los Angeles:**
  Clinical Trial for Checkpoint Inhibition in Pediatric High-Grade Glioma:
  The goals of this project are to understand how metastatic melanoma develops resistance to therapies in distinct anatomical sites, particularly the brain microenvironment.

- **National Institutes of Health (NIH) and National Cancer Institute (NCI) R01CA236910**
  Metastatic Clonal Heterogeneity and its Impace on melanoma Therapeutic Resistance:
  The goals of this project are to understand how metastatic melanoma develops resistance to therapies in distinct anatomical sites, particularly the brain microenvironment.

- **National Institutes of Health (NIH) and National Cancer Institute (NCI) R01CA260886:**
  Use of CTEP portfolio compounds to counteract phenotype conversion in GBM:
  This project will study novel combination therapies against glioblastoma.

- **National Institutes of Health (NIH) and National Institute of Neurological Diseases and Stroke (NINDS) R01CA281682:**
  Utilizing Radiation-Induced Multi-potency to Increase the Efficacy of Radiotherapy:
  The goal of this study is to utilize radiation-induced multi- potency to drive glioblastoma cells into a terminally differentiated, neuron-like state, thereby preventing tumor recurrence.

- **California Institute of Regenerative Medicine DISC2-14083:**
  Development of novel small molecules against cancer stem cells in solid cancers:
  This project seeks to develop novel multi-kinase inhibitors against glioblastoma.

- **American Cancer Society- CSCC-Team-23-980262-01-CSCC:**
  Understanding radiation-induced phenotype conversion in order to target glioma stem cells:
  This project will use DNA barcoding and single cell RNA sequencing and ATAC sequencing to understand the cellular source and molecular steps in phenotypic conversion to vascular and cancer stem cells.

Other Organizations Involved as Partners

Organization Name: Siemens Healthineers
Location of Organization: Erlangen, Germany
Partner's Contribution to Project: In-Kind and Collaboration Support. Siemens has provided an onsite engineer (Xiaodong Zhong, Ph.D.) to help with this project and others.

Organization Name: NMR Laboratory, Neuromuscular Investigation Center, Institute of Myology
Location of Organization: Paris, France
Partner's Contribution to Project: Collaboration support. Dr. Alfredo Lopez has been collaborating with us and helping us troubleshoot issues with our pulse sequence source code.

8. Special Reporting Requirements

Nothing to report.

9. Appendices

Nothing to report.