Keto Brain: Investigating the Use of Ketogenic Diets in Brain Metastases

The Ohio State University

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1. BRAIN METASTASES

Neurological tissues are among the most common (>10% of cancer patients) and debilitating sites for metastatic disease to develop (1**)**. The brain is a 'sanctuary site' for many cancer cells and remains a challenging site to treat. Surgery and radiation therapies are the most common treatments. We hypothesize that the metabolic adaptations associated with a well-formulated ketogenic diet that induces nutritional ketosis will significantly improve the response to surgery and radiation in patients with brain metastases.

2.0 STANDARD OF CARE RADIOTHERAPY

Over 200,000 patients a year will be diagnosed with brain metastases (BM) and the incidence of BM is predicted to grow due to improvement in survival with newer systemic therapies ***effective for various primary cancer diseases***. (*2*) Overall survival has also improved significantly over the years for patients with BM, so the morbidity of radiation is becoming more important. Whole brain radiation therapy (WBRT) is the most common treatment for BM to date. The majority of patients treated with WBRT suffer neurocognitive deterioration by 6 months. RTOG 0614, randomized trial of WBRT +/- memantine, found patients treated with WBRT alone had an 80% rate of cognitive deterioration at 6 months. (*3*) For WBRT we can reduce neurocognitive decline by using IMRT to spare the hippocampus, but even with HA, 50% of the patients will still develop neurocognitive failure based on NRG-CC001. Nonetheless, for patients who present with extensive (>4 lesions) BMs, WBRT is considered the standard approach and the latent challenge is to both control intracranial disease but simultaneously preserve neurocognition and prevent decline in quality of life. Moreover, SRS is the standard of care for patients with limited number (1-4) of BM. RTOG 1270 showed similar overall survival but less neurocognitive decline at 6 months using SRS vs. WBRT with patients up to 4 BM. (*4*) The obstacle in the use SRS for extensive BM is the time under treatment if each lesion is treated with a separate radiation plan. Therapy choices for this population are currently guided by the available technologies and little to no relevant information on normal tissue toxicity exist. Specifically, in vivo mapping of ultrastructure damage is still unrevealed in the context of BM. Here we have chosen to select BM patient candidates for SRS who are willing to adopt either a ketogenic or guidelines-based mixed diet to explore the clinical course of disease following radiation treatment. We purposely chose a highly sensitive cohort to study the overlapping benefits of using advanced radiation delivery, the SRS, in synergy with the potential neuro rejuvenating aspects of nutritional ketosis. We hypothesize that a sustained ketogenic diet has neuroprotective effects that can aid in neurocognitive preservation in patients treated with SRS.

3.0 Metabolic Adaptations to a Ketogenic Diet and Relevance to Metastatic Cancer

A ketogenic diet causes accelerated fat metabolism, which results in production of specific metabolites called ketones. The principal ketone body beta-hydroxybutyrate (BOHB) is now recognized as both a preferred metabolite for the brain and other extrahepatic tissue as well as a potent signaling molecule with epigenetic effects that favorably affect cellular function (*5*, *6*). Over the last 15 years, our research group has established the superiority of very low-carbohydrate ketogenic diets over traditional low-fat diets in managing insulin resistant conditions (*7*, *8*), which now includes over half the adults in the US (*9*). The metabolic state of nutritional ketosis is associated with a robust shift to almost exclusive reliance on fatty acids and ketones for fuel. Interestingly, highly competitive national caliber ultra-endurance athletes are also increasingly switching to ketogenic diets. We recently published the first paper showing that keto-adapted athletes have extraordinary fat burning capabilities at least 50% higher than the highest rates ever recorded (*10*). The reduced reliance on carbohydrate oxidation and insulin- mediated glucose

uptake has beneficial effects on satiety, weight loss, insulin sensitivity and glycemic control. There is also decreased inflammation and oxidative stress, improvements in cholesterol and lipoprotein profile, fatty acid composition, and overall cardio-metabolic risk.

Importantly, the state of nutritional ketosis should provide a favorable environment to nourish the body while deterring tumor growth through less glucose flux, insulin stimulation, and inflammation. One central tenant of tumor metabolism is an almost exclusive uptake and utilization of glucose for energy derivation. An ability to control blood glucose and insulin, without additional medication, may inherently decrease tumor viability. The reliance on glucose as a fuel substrate is exploited via 18- flourodeoxyglucose positron emission tomography (FDG-PET), the gold standard for cancer diagnosis and prognostic characterization. Evidence that targeting insulin sensitivity and improved glucose management is beneficial to cancer comes from the recent discovery that metformin, the most widely used anti-diabetic medication to improve insulin sensitivity, results in decreased risk of cancer occurrence (*11*). Nutritional ketosis resulting from a ketogenic diet consistently reduces circulating plasma glucose levels and increases insulin sensitivity (*12-14*).

Only a few human studies have investigated the effects of a ketogenic diet on cancer. Two small clinical trials that investigated the effectiveness of a ketogenic diet in advanced stage cancer patients both demonstrated slowed disease or partial remission in several patients (*15*, *16*). A recent study reported that a patient with breast cancer with PET avid metastasis in both lung and bone experienced complete remission after adoption of a ketogenic diet (*17*). The same authors further demonstrated that 60% of the adopters experienced improvements in tumor biology or prognosis, with diet adherence associated with better outcomes (*17*). ***These findings provide a strong scientific rationale for a larger and tightly controlled study in advanced cancer patients that incorporates more sophisticated methods of inducing and monitoring nutritional ketosis.***

It is well understood that cancer results in a state of chronic inflammation, in which the tumor thrives. Ketogenic diets have clinically demonstrated improved systemic inflammation status, specifically decreased concentrations of several proinflammatory cytokines (e.g., IL-6 and IL-8) (*18*), both of which are significantly increased in advanced stage breast cancer patients (*19*). This may significantly improve patient well-being and quality of life, as cancer related fatigue in patients with breast cancer has been linked with chronically elevated concentrations of the pro-inflammatory cytokine IL-6 (*20*). IL-6 increases have also been linked with an induction of the genes SNAIL and TWIST. SNAIL and TWIST are two of the major genetic regulators of epithelial-mesenchymal transition (EMT), the initial step of metastasis (*21*). Nutritional ketosis reduces the amount of cancer stem cells, a cell population that is theorized to be the root cause for metastasis and cancer recurrence (*22*). Further *in vivo* evidence has demonstrated the ability of a ketogenic diet to improve mood of individuals undertaking a weight loss program (*23*). *In vitro* research has demonstrated the ability of ketosis to decrease tumor viability and improve survival time (*24*).

One potential pathway for the discord in energy metabolism of cancer may result from PTEN and phosphoinositide 3-kinase (PI3K) pathway mutations. Within the past two decades the tumor suppressor capabilities of the phosphatase and tensin homologue (PTEN) gene have been well documented (*25*). Either PTEN inhibition or PI3K activation via insulin-mediated processes causes metabolic deregulation and result in a Warburg-like Effect. Loss of PTEN results in phosphatidylinositol-3,4,5-triphosphate dephosphorylation, as well as an increased phosphorylation of AKT via PDK1 further activating and enhancing the PI3K/AKT pathway. Since

insulin is a primary activator of the PI3/AKT pathway, tumor growth could reasonably be inhibited by therapeutic interventions that lower insulin concentration and action. One of the fundamental adaptations associated with a ketogenic diet is decreased insulin concentration and signaling, increased reliance on fatty acids and ketones as energy substrates, and dramatically reduced reliance on glucose uptake and oxidation (*10*, *26*). Fatty acids and ketones do not require insulin-dependent PI3K signaling for transport and oxidization in cells, and thus should result in decreased action of PI3K and downstream effectors including mTOR inhibition (*22*).

Aside from direct effects on tumor-based outcomes, nutritional ketosis may counteract undesirable side effects associated with drugs commonly used to manage different types of cancer. In efforts to combat inflammation associated with chemotherapeutic agents, patients are frequently administered glucocorticoids. Glucocorticoids function by binding the glucocorticoid receptor, activating transcription factors, and effecting target genes (*27*). While high doses glucocorticoids strengthen anti-inflammatory defenses and combat adverse reactions to chemotherapeutic drug infusion, they promote insulin resistance and weight gain (*27*). In fact, approximately two-thirds of individuals with a high dose of glucocorticoids exhibit hyperglycemic conditions (*28*). Glucocorticoid-induced insulin resistance manifests in a similar fashion to that of Type II diabetes. The resultant hyperglycemia is associated with increased proteolysis, *de novo* lipogenesis, and hepatic fatty acid accumulation. These unfavorable metabolic outcomes to glucocorticoids are targeted by ketogenic diets (*7*, *8*). Thus, nutritional ketosis would be expected to decrease the need for glucocorticoids owing to its anti-inflammatory effects and decreased dependency on glucose metabolism. In more aggressive cases, nutritional ketosis may also permit the use of higher doses of glucocorticoids, when needed, by mitigating untoward side effects.

Thus, there are several metabolic adaptations to a ketogenic diet that should benefit patients with brain metastases including decreased fat mass, decreased glucose flux into tumors, less insulin burden, less inflammation and oxidative stress, improved tolerance to chemotherapy and radiation, and mitigation of side effects to medications.

3.1 Non-Metabolic Roles of Ketones and Relevance to Cancer

A defining feature of a well-formulated ketogenic diet is that circulating levels of BOHB increase by an order of magnitude. A remarkable new perspective on BOHB was published in Science just a few years ago (*29*). This paper showed that BOHB is a potent histone deactylase (HDAC) inhibitor and regulator of a group of genes that protect cells from oxidative stress. Specifically, it was demonstrated that at physiological levels characteristic of nutritional ketosis, BOHB switched on specific genes that protect cells from free radical damage. Shortly after this research was published, others reported that the same mechanism of action by BOHB also potently and directly reduced insulin resistance. Since oxidative stress is prominent in the pathophysiology of aging, it has been hypothesized that BOHB may be a longevity metabolite, which is now supported by two recent papers (*5*, *30*).

It is highly probable that the nontoxic, epigenetic, drug-like effect of naturally-produced BOHB on HDAC inhibition has relevance to cancer metabolism and management. HDAC inhibitors are currently used as novel anti-cancer agents, and have been shown to arrest tumor growth and induce apoptosis in cancer cells in vitro and in vivo (*31*). Paclitaxel (taxol) is used as a first line standard of care chemotherapeutic drug. Two recent papers (*31*, *32*) demonstrated a potent synergistic effect of combining paclitaxel with an HDAC inhibitor on two different cancer cell lines. The HDAC inhibitor used in these studies was phenethyl isothiocyanate (PEITC), found in a wide variety of

cruciferous vegetables with much less relative potency compared to BOHB. Further evidence demonstrating a therapeutic role of HDAC inhibitors has been the clinical success of Vorinostat in combating triple negative breast cancer and sensitizing the malignancy to more common treatments (*33*). Vorinostat is a broad spectrum HDAC inhibitor that selectively targets similar HDACs as BOHB (*29*, *33*). Thus it may be likely that a ketogenic diet provides a non-toxic pathway for improving patient-related outcomes and increase tumor sensitivity to radiation. In our proposed study, we will customize the diet such that we optimize ketones in the same range shown to result in HDAC inhibition (*29*). We expect this unique synergistic combination will yield overall favorable patient effects (e.g., less tumor growth, decreased treatment dose to maintain effectiveness, decreased toxicity of treatment).

3.2 Current Knowledge of Ketogenic Diets in Cancer

There currently exist several basic science papers and animal studies pointing to positive effects of ketogenic diets in different types of cancers (*24*, *34*, *35*), and this is now moving into human clinical trials. Our current on-going Keto-CARE trial is the world's first and only clinical trial investigating the feasibility and efficacy of a well-formulated ketogenic diet in women with metastatic breast cancer. To date, this trial has demonstrated high levels of tolerance and feasibility as evidenced by mean blood ketones above 0.5mmol, the threshold for nutritional ketosis. Two previous studies also found a high degree of feasibility of the ketogenic diet in human patients with advanced stage cancers (*15*, *16*). Both research teams successfully demonstrated that a ketogenic diet is feasible and well tolerated with no adverse events in advanced stage cancer patients. In a cohort analysis Fine (2012) demonstrated that participants with the highest ketosis levels exhibited either stable disease, or partial remission. While findings were promising, the sample and effect sizes were small, the diets lacked sophistication and were at the lower end of nutritional ketosis, and they were not done in combination with chemotherapy. The patients studied in Fine (2012) were also later stage 4 and thus represented a population that may be more difficult to treat effectively than patients initially diagnosed with BM. A larger study with better control over dietary parameters is needed to determine the response to a ketogenic diet in patients with brain metastases. Despite the sample size limitations, interpretation of the Fine (2012) study reveals that the highest responders to ketosis were most likely to succeed. This finding corroborates results from Schmidt (2011) that showed participants who completed the intervention had higher levels of stable disease or partial remission. There currently exist approximately ten registered clinical trials investigating a ketogenic diet in cancer patients, the majority of which are focused on head, neck, lung and brain primary tumor sites. None of these trials are investigating patients with brain metastasis. Due to the unique etiology and most common tumorigenic pathways, brain metastasis presents a myriad of potential therapeutic mechanisms through which ketosis may target.

Thus, the next logical step is to perform a larger study in patients with brain metastasis that incorporates principles of a personalized well-formulated ketogenic diet. Our research group includes experts in ketogenic diets and medical and radiation oncologists. Thus, we are uniquely suited to perform such a study.

4. RATIONALE AND FEASIBILITY

To date, numerous preclinical studies have demonstrated the ability of nutritional ketosis and caloric restriction to augment the therapeutic efficacy of radiotherapy (*36*, *37*). Use of a ketogenic diet is known to decrease the protein expression of HIF-1α and VEGFR2, and may increase radio

sensitivity by normalizing tumor vasculature and increasing facilitated oxygen delivery to tumor cells. Several additional proposed mechanisms exist for enhancing radiosensitivity of the malignancy including: HDAC dependent mechanisms, ATP deprivation, mitochondrial ROS production and downregulation of IGF-1 receptor. Short-term fasting, a way to increase ketones acutely, has been demonstrated to speed up mitotic rates and thus facilitate a DNA damage sensitization. Lastly, a recent study showed that glucose restriction lowered Ki-67 expression, clonogenic frequency and rate of proliferation in gliomaspheres *in vitro*.

Our research team has an established expertise in conducting ketogenic diet interventions. Many individuals have adopted a low-carbohydrate diet for health reasons, yet there is scarce professional support available to provide guidance and support, especially for ketogenic diets. We have scientific expertise and practical knowledge of both ketogenic and current standard of care cancer diets combined with a passion to empower people with the tools to implement these eating approaches into their lifestyle. This project is highly patient-centered. We will support patients who are randomized into either one of the intervention groups and provide them with a personalized eating plan designed to have maximal therapeutic impact and positively impact their lives. To that end, this project is unique in that it is highly patient-centered while also designed to have a substantial scientific and practical impact on medical management of brain metastasis treatments.

5.0 OBJECTIVES

We propose to conduct a highly controlled feeding study for 16 weeks to examine the effects of an individualized ketogenic or American Institute of Cancer Research (AICR) dietary approach on tumor response to targeted radiation therapy, as well the neural preservation in non-targeted areas of the brain. Our specific aims are as follows:

Primary objective: Assess the feasibility of maintaining a ketogenic versus AICR diet in patients with brain metastasis selected for radiosurgery. Endpoints:

- Circulating glucose (primary endpoint)
- Circulating ketones (primary endpoint)
- Retention
- Side-effects

Secondary objective: Assess the preliminary efficacy of the ketogenic versus AICR diet on tumor response to targeted radiation therapy. Endpoints:

- RECIST v. 1.1 will be utilized to evaluate responses to therapy

Exploratory objective: Assess the preliminary effects of the ketogenic versus AIRC diet on cognitive function and quality of life in BM individuals undergoing radiation therapy.

6.0 STUDY DESIGN

This pilot study will be a single center, randomized controlled study of 24 participants with diagnosed BM (various primary disease sites) comparing the effect of a ketogenic (n=12) and AICR (n=12) diet. Randomization will be balanced by blocks of random sizes but no stratification due to the small sample size. Both groups will undergo a 16-week diet intervention. In an effort to maintain a patient centric focus and monitor changes in quality of life (QOL) all patients will complete psychosocial and behavioral inventories. These inventories aim to capture a holistic view on the proposed nutritional intervention during treatment. Primary outcomes will be determined at baseline, 8 weeks, and 16 weeks (see section 13 – Study Calendar) while patient-centric outcomes will be assessed every four weeks.

6.1 Eligibility Criteria

Inclusions:

- Ages 18-75 years old
- Measurable brain lesions noted on baseline MRI imaging
- Graded Prognostic Assessment > 1.5
- Body mass index (BMI) ≥18 kg/m^2
- Eastern Cooperative Oncology Group (ECOG) Performance status of 0-1 (0=participant has either normal activity, 1= participant has some symptoms but is nearly full ambulatory)
- Able and willing to follow prescribed diet intervention
- Scheduled to receive SRS

Exclusion criteria:

- Undergoing whole brain radiation therapy
- BMI <18 kg/m^2
- Pregnant or nursing women
- Not willing to be randomized into either of the dietary interventions
- Unable to provide Informed Consent
- No previous diagnosis of small cell lung carcinoma
- No previous or suspected leptomeningeal disease
- Type 1 diabetes or insulin-dependent Type II diabetes
- Abnormal renal function (GFR < 55 mL/min, creatinine >2.0, urinary albumin >1 g/day)
  Not MRI eligible

7.0 TREATMENT PLAN

Participants will have counseling by the attending physician for additional applicable medications for any treatment related side effects or toxicities. The intervention groups will undergo their randomized dietary regimen for 16 weeks.

7.1 Treatment considerations

7.1.1 Prior to Treatment

After informed consent is obtained from the patient and prior to randomization, baseline QOL and functional independence assessments must be completed.

The SRS dose has been selected in order to provide a high rate of local control with minimum risk of radionecrosis. The SRS dose is decreased modestly for larger lesions in order to account for the volume effect on complication rates. While prior SRS to other lesions is allowed, repeat SRS to the same lesion/location is NOT allowed.

Treatment Timing: Patients must initiate radiosurgery treatment ≤14 days after registration. Treatment should occur within 14 days of the MRI for treatment planning.

Cytotoxic Chemotherapy is not allowed 3 days before SRS, the same day as the SRS, or 3 days after the completion of the SRS.

7.2 Stereotactic Radiosurgery (SRS) to Surgical Bed Guidelines: (Fractionated SRS)

For unresected brain metastases see Section 7.3.

For unresected brain metastases see section 7.3. If all lesions cannot be treated on the same day, all lesions MUST be treated with ≤8 days of completing treatment of the first legion.

For any questions regarding dose and volumes physicians may contact the study radiation oncology PI, Joshua Palmer, for guidance

For unresected brain metastases see Section 7.3. If all lesions cannot be treated on the same day, all lesions MUST be treated within ≤8 days of completing treatment of the first lesion.

7.2.1 Medications

Patients may be given a short steroid taper over 1-2 weeks, 2-4 mg of dexamethasone twice daily for 5 days, then 2-4 mg daily for 5 days. Concurrent use of a proton pump inhibitor or H2 receptor antagonists are encouraged if on steroids for an extended period.

7.2.2 Equipment

Modality: Gamma knife or X-rays with nominal energy of 4 megavoltage (MV) or greater for accelerator-based treatments, including isocentric conical collimators, mini-multi-leaf (5 mm or less) technology, single isocenter mutli-target technique, or linear accelerators mounted on robotic arms will be used.

7.2.3 Target Volume Definitions

The volumes shall be defined by a planning MRI brain scan. ICRU-91 and AAPM TG-263 nomenclature target volumes are defined as follows and will include laterality and tumor location (ie. GTV(#)_frontal_L and PTV(#)_cerebellum_R):

- Gross Tumor Volume (GTV): Pre-operative MRI post-contrast volumetric imaging, post-operative MRI post-contrast volumetric imaging and CT simulation or treatment planning MRI of the brain will be used. The surgical bed is defined as the entire surgical cavity noted on T1 post-contrast volumetric imaging, including blood products and postsurgical changes if these are limited to the surgical bed. For deep lesions this does not include the entire surgical tract through the brain. In addition, this does not include edema. However, the GTV will include the adjacent meningeal surfaces which were shown on the pre-operative MRI imaging and any adjacent meningeal surfaces adjacent to the resection bed. GTV will not extend outside of the calvarium (CT simulation bone window is preferred if available).
- Clinical Tumor Volume (CTV): This is defined as the GTV with a 2 mm margin as seen on planning MRI. However this 2 mm margin does not need to expand into structures that typically are not at risk of tumor infiltration from brain metastases such as air or bone.
- Planning Target Volume (PTV): Typically, PTV will equal CTV with no expansion. However, an optional 1mm expansion of CTV is allowed when defining PTV.
- Contouring will be reviewed by the radiation oncology PI, Joshua Palmer.

7.2.4 Target Dose:

Prescription Specification: The dose should be prescribed to the highest isodose line encompassing the PTV (surgical cavity plus 2 mm – see section 7.334), which can range from 45% to 95% of the maximum dose.

Dose Definition: Dose is specified in Gray (Gy).

Prescription Dose: The total prescribed dose is determined by the GTV. The volume determines dose due to the often irregular shape of surgical cavities:

Fractionated SRS
Lesions <30 cc receive 27 Gy /3 fractions
Lesions ≥ 30 cc to < 5 cm receive 30Gy /5 fractions

Dose Conformity: The ratio of the prescription isodose volume to the target volume **should be** between 1.0 and 2.0. Preferably, the conformity index should be between 1 and 1.2. It is

understood that this ratio may be difficult to achieve with some very small lesions. For lesions less than 5 mm in size, a ratio up to 3.0 is acceptable.

7.2.5 Treatment Technique

An immobilization/patient localization system is mandatory for this study. Single isocenter, multi-target, multiple isocenter and non-isocentric techniques are permitted.

7.2.6 Normal Tissue/Critical Structures

The treatment parameters should be modified to optimize the fit of the prescription volume to the target volume while minimizing dose to critical structures. Normal Tissue tolerances are based on *AAPM TG 101*.

- **3 (three) Fraction** Dose Constraints. The maximum point dose to the optic pathway should be less than 17.4 Gy, and <0.2cc will receive 13.8 Gy. No more than 0.5 cc of the brain stem should exceed 18 Gy, maximum point dose 23.1 Gy.
- **5 (five) Fraction** Dose Constraints. The maximum point dose to the optic pathway should be less than 23 Gy, and <0.2cc will receive 20 Gy. No more than 0.5 cc of the brain stem should exceed 23 Gy, maximum point dose 28 Gy.

7.3 Guideline for Unresected Brain Metastases

If all lesions cannot be treated on the same day, all lesions MUST be treated within ≤10 days of treatment of the first lesion.

7.3.1 Medications

If single fraction SRS, patients may be given an intravenous bolus dose of 4 to 16 mg of dexamethasone or 40 to 80 mg of SoluMedrol at the time of SRS, at the discretion of the treating physician. Patients in both arms may alternatively be given a short steroid taper over 1-2 weeks, 2-4 mg of dexamethasone twice daily for 5 days, then 2-4 mg daily for 5 days. Concurrent use of a proton pump inhibitor or H2 receptor antagonists are encouraged while on steroids. See Section 8.1 for ancillary/concomitant therapy.

7.3.2 Equipment

Modality: Gamma knife or X-rays with nominal energy of 4 megavoltage (MV) or greater for accelerator-based treatments, including isocentric conical collimators, mini-multi-leaf (5 mm or less) technology, single isocenter mutli-target technique, or linear accelerators mounted on robotic arms.

7.3.3 Target Volume Definitions

The volumes shall be defined by typical standard of care MRI brain scan (recommended MR protocol included in Appendix B: IMAGING). ICRU-91 and AAPM TG-263 nomenclature target volumes are defined as follows and will include laterality and tumor location (ie. GTV(#)_frontal_L and PTV(#)_cerebellum_R):

- Gross Tumor Volume (GTV): This is defined as the contrast enhanced tumor seen on planning MRI. The maximal cross-sectional diameter must be < 3.0 cm.
- Clinical Tumor Volume (CTV): The CTV will equal the GTV with no expansion.
- Planning Target Volume (PTV): This is defined as the CTV for this study. Typically there will be no expansion of CTV to create PTV, but an optional 1-2 mm expansion of CTV is allowed when defining the PTV determined by the treating physician based on immobilization and treatment technique.

7.3.4 Target Dose:

Prescription Specification: The dose should be prescribed to the highest isodose line encompassing the PTV, which can range from 45% to 95% of the maximum dose.
Dose Definition: Dose is specified in Gray (Gy).
Prescription Dose: For unresected metastases the fractionation schedule should match the fractionation schedule for the surgical bed as treating all lesions at the same time.

Single Fraction SRS. The total prescribed dose is determined by treatment arm and tumor size (maximal diameter).

SSRS Unresected Metastasis:
Lesions < 1.0 cm receive 24 Gy
Lesions ≥ 1.0 to ≤ 2.0 cm receive 22 Gy

Three Fraction SRS.

FSRS Unresected Metastasis:
Lesion >2.0 cm receive 27 Gy /3 fractions

Five Fraction SRS.

Arm B (FSRS) Unresected Metastasis:
Lesion >3.5 cm receive 30Gy /5 fractions

Dose Conformity: The ratio of the prescription isodose volume to the target volume (PTV) should be between 1.0 and 2.0. It is understood that this ratio may be difficult to achieve with some very small lesions. For lesions less than 5 mm in size, a ratio up to 3.0 is acceptable.

7.3.5 Treatment Technique

An immobilization/patient localization system is mandatory for this study. Single isocenter, multitarget, multiple isocenter and non-isocentric techniques are permitted.

7.3.6 Normal Tissue/Critical Structures

The treatment parameters should be modified to optimize the fit of the prescription volume to the target volume while minimizing dose to critical structures. The dose constraints depend on the fractionation schedule utilized.

- Single (one) Fraction Dose Constraints. The maximum point dose to the optic pathway should be less than 9 Gy. No more than 1cc of the brain stem should exceed 12 Gy.
- 3 (three) Fraction Dose Constraints. The maximum point dose to the optic pathway should be less than 17.4 Gy, and <0.2cc will receive 13.8 Gy. No more than 0.5 cc of the brain stem should exceed 18 Gy, maximum point dose 23.1 Gy.
- 5 (five) Fraction Dose Constraints. The maximum point dose to the optic pathway should be less than 23 Gy, and <0.2cc will receive 20 Gy. No more than 0.5 cc of the brain stem should exceed 23 Gy, maximum point dose 28 Gy.

Please refer to the table in Section 7.2.6 for the standard naming convention for critical structures.

Dietary Interventions (16 weeks): Four to seven days prior to the baseline testing, participants in both the Ketogenic and AICR Diet groups will be provided with meals consistent with their diet group that will provide 100% of caloric needs to assist participants with their dietary transition and to ensure participants in the ketogenic arm get into nutritional ketosis by the time of baseline treatment. Food for both diet groups will be provided by a 'ready to eat' meal delivery company along with supplemental snacks. After baseline testing both groups will be provided individualized counseling and support to follow guidelines for each respective eating pattern to maintain their respective diet for the remainder of the study. The Ketogenic Diet will consist of <50 g carbohydrate (personalized based on level of ketones checked daily by finger stick), ~15-20% protein and ~70-75% fat. Total energy intake will be *ad libitum* to permit overweight/obese participants to restrict caloric intake to induce weight and fat loss for those who are overweight. Participants will be provided with a handheld glucometer (Precision Xtra, Abbott Nutrition) and ketone test strips in order to check the concentration of BOHB from a finger stick. Normal levels of ketones in a person consuming more than 100 grams of carbohydrate per day is <0.1 mmol/L. The goal of the diet will be to induce a state of nutritional ketosis defined as blood ketones >0.5 mmol/L. We believe that a level of ketones above 1.5 mmol/L will enhance the metabolic therapy and deliver better outcomes based on preliminary studies in women with breast cancer (Fine et al., 2012; Schmidt et al., 2011) and blood BOHB concentrations shown to inhibit histone deacetylases (Shimazu et al., 2013b).The carbohydrate level required to induce nutritional ketosis will vary from person to person and thus objective feedback provided by testing blood ketones is a novel tool we will use to personalize the diet by titrating the carbohydrate and protein intake to the participant's individual ketosis threshold. We will encourage consumption of a wide range of ketogenic-appropriate whole foods including non- starchy vegetables, fruits (berries, olives, tomatoes, lemons/limes), meats (beef, chicken, pork, fish, lamb), nuts and seeds, oils (olive, canola, coconut), cheese, butter, cream, and eggs. Contrary to the misconception that the diet is boring or overly restrictive, it is noteworthy that even the most carbohydrate intolerant person can choose from a wide range of whole foods including berries and a wide assortment of vegetables.

Participants randomized into the AICR arm will follow the "Model plate for a cancer preventative diet" which is outlined as follows: "Aim for meals made up of 2/3 (or more) vegetables, fruits whole grains or beans and 1/3 (or less) animal protein. If you are overweight, consider gradually reducing that number. Controlling portion size at home and in restaurants makes a long-lasting difference in controlling your weight."

Adherence and Retention: Achieving good adherence and retention is one of the most challenging aspects of prospective diet intervention studies. Most studies of low- carbohydrate ketogenic diets have poor compliance because of poor understanding of proper formulation and implementation challenges. Historically we have had excellent compliance and satisfaction to a ketogenic diet in both feeding and free-living studies. Ketogenic diets should not be forced on people so we carefully explain details of the diet (including meal plans, acceptable and non-acceptable foods, etc.) so that individuals are knowledgeable about what to expect and can make an informed choice whether to enroll in a study. We have performed multiple ketogenic feeding experiments including a recently completed study at OSU where we observed excellent adherence as determined by daily capillary BOHB measurement over a 10-week period and another ongoing trial in patients with metastatic breast cancer. The proposed study will be the first time we work specifically with cancer patients who are undergoing radiation therapy. We acknowledge there may be unique challenges in regard

to compliance in this population of patients with metastatic cancer due to their cancer diagnosis and/or drug-induced effects on appetite and preferences for food.

Due to the small sample size proposed (n=24), we will have an enhanced researcher/participant relationship to promote individualized education and counseling to facilitate nutritional ketosis and perceived benefit. We have developed extensive educational materials and resources to help participants successfully make the behavior change to eating a very low-carbohydrate diet and an AICR diet. Well-formulated ketogenic diets are unique when compared to other diets in that there exists an ability to receive direct feedback on participant adherence via finger prick and handheld glucometer that measures the biomarker BOHB. Enrolled participants will be in daily correspondence with research staff throughout the duration of the study to provide a personalized nutrition approach and optimize the amount of time spent in nutritional ketosis and to provide continued support and education for the AICR cohort.

8.1 Clinical assessments

Standard of Care Clinic Visits
Participants will be evaluated in the clinic prior to the start of therapy and study intervention, as well as at monthly intervals for history, physical examination for vitals, height, and weight. Hearth Hope Index (HHI), SF-36, cancer specific FACT-surveys, Brief Pain Inventory, and Brief Fatigue Inventory, and self-report diaries (adverse events, adherence and palatability surveys) will be completed to evaluate QOL and will be administered at baseline, monthly during the intervention and at study terminus. Serum or plasma-based biological markers will also be collected at baseline, and midpoint and study terminus. Standard of care brain imaging will be utilized at baseline, 8 and 16 weeks to evaluate disease status and progression. Body composition and bone mineral density will be assessed at baseline and 16 weeks using dual energy x-ray absorptiometry (DXA).

8.2 *Adherence*
Participant adherence with the study intervention will be encouraged and monitored in several ways. All participants will be provided a handheld glucometer (Precision Xtra, Abbott Nutrition) and reagent strips that measure BOHB from a small amount of blood obtained by finger stick. Although there is variability between people, BOHB levels in individuals consuming more than 100 grams of carbohydrate per day are usually ≤0.1 mmol/L. A primary goal of the initial diet intervention is for each participant to enter nutritional ketosis, which starts at 0.5 mM and extends up to approximately 3.5 mmol/L. This is a strong indication that fat is being used as one's primary fuel, and there may be other therapeutic benefits associated with ketones in this range. Transient increases in ketones above 3.5 mmol/L may occur after exercise, but regulatory feedback systems keep ketones from elevating to dangerous levels that are seen in uncontrolled type-1 diabetics who have insufficient insulin (i.e., >10 mmol/L). Participant's information will be kept private and only made available to IRB approved research staff. During the intervals between research- based visits participants will report their ketone levels daily to the research coordinator and lead RD on the team and adjustments to the diet made accordingly.

We will counsel patients to aim for a level > 1.0 mM while following principles of a well-formulated ketogenic diet. If ketone readings are lower than 0.5 mM, we will individually work with the patient to identify appropriate changes to their diet/lifestyle to achieve nutritional ketosis. The monitoring of daily ketones for patients on the ketogenic diet provides a gold-standard biofeedback marker that is objective and quantifiable for adherence. Deviation from the dietary standards of a well-formulated ketogenic diet will cause rapid decreases in ketones and elevations in blood glucose,

which will be identified in the patient:researcher interaction. For the standard diet arm, this will present a greater challenge. We will ask participants to inform us of dietary deviations and these will be identified and logged. The monthly self-report diaries will be used for tracking any minor adverse effects (GI distress, bloating, gut distension, diarrhea etc.). If participants do not experience any side effects, it is possible they will not have a self-report diary to share monthly.

8.3 Data and Records

Primary source documents will include forms routinely used at the James Comprehensive Breast Center, namely the Breast Patient Information Form, clinic and office notes as well as laboratory and radiology reports.

8.4 Safety Monitoring

Adverse events will be monitored by self-reporting of signs and symptoms. Patients will maintain weekly contact with researchers and any possible ill effects will be noted and discussed with the attending physician or Research Nurse to discuss and manage any possible side effects. Patients will be counseled with regard to potential signs of treatment toxicity and should immediately contact the PI, study coordinator or treating physician in the event such a problem arises.

8.5 Accountability

All interventional testing will be provided at no cost to the patient. Further, as a result of the time-intensive nature of the study participants in the intervention group will receive a completion stipend of $500.

9.0 BIOCHEMICAL, COGNITIVE AND PSYCHOSOCIAL TESTING

Activity Monitor:

Physical activity during a course of radiation therapy can be associated with decreased fatigue and may potentially mitigate other side effects. An activity tracker will be worn around the wrist daily for heart rate and step counting.

Peripheral Blood Samples:

Additional lab samples, which are not part of standard care, will be obtained at baseline and paid for as a research cost. Blood will be collected at baseline and every 8 weeks throughout the study duration until terminus.

Continuous Glucose Monitoring (CGM)/ Continuous Ketone Monitoring (CKM):

In order to more accurately quantify the glycemic control and diet adherence of patients using either the AICR or Ketogenic diet we will use three 10-14 day recording periods of CGM/CKM at baseline, midpoint and post-testing. A small Bluetooth compatible unit will be affixed to either the triceps or adipose located near anterior superior iliac spine. CGM/CKM will be analyzed for glucose excursions, mean glucose levels and other markers of glycemic control.

Insulin Resistance and Pre-Diabetes Panels

Due to the high correlation between insulin resistance, diabetes and breast cancer occurrence/prognosis, we will examine the effects of the two diets on several biomarkers including HbA1c, Insulin, Glucose, and Sex hormone binding globulin (SHBG).

Inflammation Panel

Due to the fact that anti-cancer therapies result in increased inflammation, we will examine a panel

of inflammation markers. Each of the following markers is associated with either prognosis or quality of life. The markers to be assessed will be: TNF-α IL-6, IL- 8, CD-14, and CD-16.

Collection and Handling of Specimens
At baseline, and on a monthly basis until study terminus blood will be collected from the participant. Prior to testing all vacutainers will be labeled per study requirements. Blood will be collected into vacutainers, inverted and then stored depending on requirements for each assay.

Cognitive Testing
The current trial will build on the successes of RTOG 0614 and NRG-CC001 in utilizing the same neurocognitive function (NCF) measures with the addition of the PROMIS Cognitive Function Short Form 8a self-report assessment. There are a number of advantages of such an approach including familiarity and acceptance of these measures by clinical research staff, the well-established use and validation of these measures in brain metastasis research, and the possibility of future post-hoc analyses between studies.

The NCF tests to be used in this study (the Hopkins Verbal Learning Test – Revised (Benedict 1998), Trail Making Test (Tombaugh 2004), and the Controlled Oral Word Association (Ruff 1996) are the same tests as were used in RTOG 0614, NRG-CC001, NCCTG/Alliance N0574, and NCCTG/Alliance N107C, and that are being used in numerous ongoing brain met trials (e.g., NRG-CC003, SWOG S1827).

While NCF outcomes have been recognized as being crucial in the brain metastasis population (Lin 2013), there is also interest in evaluating the impact of treatment arm on patient-reported outcomes. PROMIS Cognitive Function Short Form 8a measures perceived cognitive abilities (e.g., memory, attention, and decision making) and the application of such abilities to everyday tasks (e.g., planning, organizing, calculating, remembering and learning). The PROMIS-8, assessment is brief and therefore not a significant burden for patients to complete.

The Hopkins Verbal Learning Test – Revised (Benedict 1998), Trail Making Test (Tombaugh 2004), and the Controlled Oral Word Association test (Ruff 1996) are widely used and standardized psychometric instruments that have been shown to be sensitive to the impact of cancer and the neurotoxic effects of cancer treatment in other clinical trials (Gilbert 2014; Meyers 2004; Wefel 2011). The tests have published normative data that account for age and, where appropriate, education and gender. The NCF assessments will be administered at baseline, 8 and 16 weeks.

The established metric for clinically-significant change is the Reliable Change Index (RCI; Jacobsen 1991). The RCI is derived from the standard error of measurement of each test and represents the 90% confidence interval for the difference in raw score from baseline to the next assessment that would be expected if no real change occurred:

RCI = 1.64(standard error of difference), where standard error of difference
$= [2(\text{standard error of measure}^2)]^{1/2}$, and standard error of measure
$= \text{standard deviation}_1[(1 - r_{xy})^{1/2}]$

This yields the following RCI values for each test in the Clinical Trial Battery:

NCF Test	RCI Value
HVLT-R Total Recall	5
HVLT-R Delayed Recall	3

HVLT-R Delayed Recognition	2
TMT Part A	12
TMT Part B	26
COWA	12

At each assessment, change in raw test score relative to baseline are calculated, and declines in a score that meets or exceeds the RCI value is categorized as a failure. **Cognitive failure (CF)** is defined as a decline on at least one of the Clinical Trial Battery tests (HVLT-R, TMT, COWA) that meets or exceeds the RCI value.

PROMIS Cognitive Function Short Form 8a v2.0: This measure is a 8-item questionnaire that assesses patient-perceived cognitive concerns over the past 7 days. The 8-item short form has demonstrated excellent internal consistency reliability and has criterion validity related evidence for measuring cognitive concerns but not cognitive performance. This measure asks the patient to rate the frequency of the following cognitive complaints over the preceding 7 days. Each question has five response options ranging in value from one to five (with lower scores indicating more severe cognitive concerns). The total raw score for a short form would be the sum of the values of the response to each question (therefore, for a short form which all questions are answered, the lowest possible score is 8 and the highest possible raw score is 40).

Collection of Psychosocial and Behavioral Inventories

Psychological effects of the combinatory therapy will be evaluated using several inventories. The Hearth Hope Index is a 12-item adapted version of the Hearth Hope Scale designed to assess feelings of 'hope' within the patient. The Short Form-36 is a 36-item patient reported survey of patient health status. Each scale is transformed into a 0-100 score and includes eight sections: vitality, physical functioning, bodily pain, health perceptions, role functioning, emotional functioning, social functioning and mental health. Brief Pain Inventory is a short form used for clinical trials that rapidly evaluates the severity of pain and impact on body functioning, and the Brief Fatigue Inventory is used to evaluate the severity and impact cancer-related fatigue. To more directly assess the emotional and behavioral response to the diet initiation and maintenance we will employ the Functional Assessment of Anorexia/cachexia Treatment. The FAACT measures the general aspects of quality of life as well as specific anorexia and cachexia related concerns. These surveys will help to determine the individual's psychosocial response to the eating changes. Additionally, participants will be asked to maintain an event diary to determine diet palatability and any adverse event reporting. Inclusion of these surveys and diaries will provide important data on the feasibility of a ketogenic diet in patients with brain mets. Expected time for completion of the surveys is approximately 30 minutes.

10 PROCEDURES FOR PATIENT ENTRY ON STUDY

This study will be open for accrual at the James Comprehensive Cancer Center at OSU. As part of the screening process, interested patients who meet initial eligibility requirements will be contacted by research staff and the specific requirements, risks/inconveniences, and other details of the research will be explained. Patients will have an opportunity to have all their questions and concerns about the research study addressed. The goal of this session is maximum understanding of the program requirements and its impact on their life. This process is designed to meet ethical obligations to the participant and improve retention by fostering a positive relationship between the participant and the research staff. The person obtaining informed consent will tell the patient that 1) participation is voluntary, 2) participation or non- participation will not affect their usual care and management, and 3) patient confidentiality will be maintained in the event that the results of the

study are published. Patients will be provided with a consent form to review, and all questions answered. After signed informed consent has been obtained, a study identification number will be assigned to the patient for use on all data collection forms and samples.

11 POTENTIAL RISKS AND MANAGEMENT

Because we will be obtaining information about a participant's medical history, lifestyle behaviors, and measuring biomarkers that will become part of the electronic health record, there is a chance that we will uncover or discover sensitive information regarding a person's health status. Although unlikely, this information could cause emotional distress, increase personal expense for treatment, or, if obtained by insurance companies or employers, could be used as justification to raise insurance rates or affect employability. To ensure privacy/confidentiality, information that is received from patients will be kept confidential to the extent allowed by law. Patient data will be entered into the electronic health record and hard copies will be kept in a secure filing cabinet on site for the duration of the study. We will assign all patients a code number to be used on forms, sample collection containers and other research materials. Subject codes will be employed for database management and when statistical analyses are performed. There will be a single key to the coded data kept on a password protected computer. Computer files containing names, addresses or other identifiers will be limited to authorized personnel at the site who have access to the computer data base using a password protected program. All investigators, professional medical staff, and technicians are aware of the confidentiality involved with the proper conduct of such a study. Consistent with the conduct of human research studies, the data will not be available or divulged to anyone outside of the experimental research team. The results from the study may be published, but will have no identifiers.

Radiation Treatment.

Possible Side Effects of Radiosurgery (Radiation)

COMMON, SOME MAY BE SERIOUS In 100 people receiving radiation therapy, 20 to 100 may have:
• If a head frame is used, temporary (short-term) pain from the head frame placement

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving radiation therapy, 4 to 20 may have:
• Headache • Tiredness • If a head frame is used, bleeding and/or infection around the head frame pin sites

RARE, SOME MAY BE SERIOUS In 100 people receiving radiation therapy, 3 or fewer may have:
• Nausea • Reddening of the skin • Localized hair loss which may be permanent

RARE, AND SERIOUS In 100 people receiving radiation therapy, 3 or fewer may have:
• Decreased brain function such as motor function (coordination/movement) • Swelling of the brain in the treated area which may require steroids • Severe local damage to or death of normal brain tissue, which may require surgery to remove • Seizure

Ketogenic Diet. There are no significant risks associated with consuming a well-formulated ketogenic diet. For patients using medication to control blood sugar and blood pressure, there is a need to reduce these medications rather quickly at the onset of the diet to prevent low blood sugar and hypotension. In this study, we will exclude those patients who have type-2 diabetes using insulin. In our prior research we have assessed thousands of metabolic panels in patients assigned to ketogenic diets. Abnormal responses are rare, but it is expected that there will be modest changes in some metabolic parameters. These markers are expected to remain within normal limits and not pose a serious concern. For example, uric acid levels often increase during the first few weeks of a ketogenic diet and then return to or below baseline after 1-2 months. This transient increase does not exacerbate gout or have other untoward effects, since the elevation is due to competition with ketones for renal excretion, in contrast to increased intracellular synthesis of uric acid. Nutritional ketosis is associated with natriuresis (increased loss of sodium in the urine) and fluid loss. If the extra sodium excreted is not compensated for in the diet, the subsequent contracted plasma volume can manifest in side effects and adrenal stress including a hormonal response that disrupts body mineral status. Our diets contain adequate sodium and potassium to ensure mineral nutriture. The diet intervention may be challenging for participants since it will require them to limit foods, they are accustomed to eating. Participants will be made aware of the general dietary requirements including lists of foods they will need to restrict (as well as foods that will be permitted) during the informational session, so they can make an educated decision to participate.

Body Composition. The DXA scan has a risk that is negligible, as the skin entrance dose of radiation due to the application of the exam is very small. In a whole-body scan, which is the mode used in this project, the skin entrance dose of radiation per scan is ~0.04 millirem. On average in the US a person receives ~0.85 millirem per day of background radiation. For another comparison, a chest X-ray delivers ~10-20 millirems per scan. Thus, the level of radiation exposure is extremely low. Since we don't know what effect the radiation could have on an unborn baby, we will perform a urine pregnancy test before the scan for all women of child bearing age in the study.

Blood Draws. Blood draws by venipuncture may cause discomfort at the puncture site and the development of a slight bruise. Participants may also experience lightheadedness or fainting during the blood draw and there is a slight risk of infection. All blood draws will be taken by trained phlebotomists. The total blood volume at each testing session will be less than 50 mL, which translates into less than 200 mL over 6 months.

Ketone Testing. Ketone testing will be done daily by finger stick using a small 26G lancet. There is slight transient discomfort associated with this procedure.

This is a diet intervention study and thus no risk for toxicity exists beyond that normally present during typical treatment. Nevertheless, we will record type of modification and toxicity management

in detail for each patient.

The severity of adverse reactions is categorized as grade 1 to grade 5 in increasing severity. General descriptors for the toxicity grades range from none to fatal:
Grade 1 – Mild (The adverse reaction does not interfere in a significant manner with the subject's normal functioning level. It may be an annoyance.)
Grade 2 – Moderate (The adverse reaction produces some impairment of functioning but is not hazardous to health. It is uncomfortable and/or an embarrassment)
Grade 3 – Severe (The adverse reaction produces significant impairment of functioning or incapacitation and is a definite hazard to the subject's health)
Grade 4 – Adverse reactions that include or lead to either a) a life-threatening event, though acute and without permanent effect, b) prolonged inability to resume usual life pattern, or c) impairment of ability to adequately deal with future medical problems
Grade 5 – Death related to AE

Toxicity will be monitored during study visits and telephone calls using the National Cancer Institute Common Terminology Criteria for Adverse Events v4.0 (CTCAE) of the National Cancer Institute will be used (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.02_2998-09-15_QuickReference_5x7.pdf). Grade 3, 4 and 5 toxicities will be reported as adverse events. Patients with Grade 3-4 adverse reactions that are related to the diet will be removed from the study.

The attribution of each toxicity will be ascertained by treating physician to both standard therapy as well as to diet intervention during the study. Treating physicians will manage suspected toxicity for dose holds and dose modifications as part of standard of care according to package insert recommendations.

12.0 ADVERSE EVENT REPORTING

12.1 Definition

Adverse event: Any unfavorable and unintended sign (including abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure, regardless of whether it is considered related to the medical treatment or procedure; also an "unanticipated problem" of any nature (e.g., psychological or social harm) (designated as unrelated, definitely related, probably related, or possibly related; see below)

Serious adverse event: Any adverse event that is fatal or life threatening, is permanently disabling, requires inpatient hospitalization or prolongs hospitalization, or results in a congenital anomaly or birth defect

Life-threatening event: Any adverse event in which the subject is at immediate risk of death from the reaction as it occurs; does not include a reaction that, if it were to occur in a more serious form, might cause death

Unexpected event: Any adverse event that is not identified in nature, severity, or frequency in the investigator brochure, study protocol, consent form, or IND application; or the event was more serious than anticipated

<u>Association:</u>

Definitely Related: An adverse event that has a timely relationship to the administration of the investigational drug/study procedure and follows a known pattern of response which no alternative cause is present

Probably Related: An adverse event that has a timely relationship to the administration the investigational drug/study procedure and follows a known pattern of response, but which a potential alternative cause may be present

Possibly Related: An adverse event that has a timely relationship to the administration the investigational drug/study procedure, follows no known pattern of response, potential alternative cause does not exist

Unrelated: An adverse event for which there is evidence that it is definitely a related cause other than the investigational drug/agent; in general, no timely relationship administration of the drug/procedure exists, or if so, the event does not follow a pattern response and an alternative cause is present

The Common Terminology Criteria for Adverse Events v4.0 (CTCAE) of the National Cancer Institute will be used. The severity of adverse reactions is categorized as grade 1 to grade 5 in increasing severity. Grade 3, 4 and 5 toxicities will be reported as adverse events. General descriptors for the toxicity grades range from mild to fatal:

Grade 1 – Mild (The adverse reaction does not interfere in a significant manner with the subject's normal functioning level. It may be an annoyance.)
Grade 2 – Moderate (The adverse reaction produces some impairment of functioning but is not hazardous to health. It is uncomfortable and/or an embarrassment)
Grade 3 – Severe (The adverse reaction produces significant impairment of functioning or incapacitation and is a definite hazard to the subject's health)
Grade 4 – Adverse reactions that include or lead to either a) a life-threatening event, though acute and without permanent effect, b) prolonged inability to resume usual life pattern, or c) impairment of ability to adequately deal with future medical problems
Grade 5 – Fatal

12.2 Documentation

All adverse events must be documented in detail within the medical record. The patient will be observed and monitored carefully until the condition resolves, stabilizes, or its cause is identified. All adverse events, including laboratory abnormalities, will be followed up according to good medical practices. Information to be recorded includes the following:

a. Specific type of reaction.
b. Duration of reaction.
c. Severity/grade of reaction according to the NCI Common Terminology Criteria for Adverse Events v4.0 (CTCAE).
d. Suspected cause of the reaction (i.e. possibly or probably related to one of the following: study treatment, progression of disease, concurrent medications, concurrent illness, or other factors).
e. Changes made in the administration of the study drugs and other actions taken to alleviate the clinical event.
f. Patient's response to medical interventions.

12.3 Reporting

According to FDA regulations (21 CFR 312.32), IND safety reports shall address "any adverse

experience associated with the use of a drug that is both serious and unexpected." The IRB will be notified of any adverse event fulfilling the following criteria:

12.3.1 The adverse event is **SERIOUS** (as defined above),
or
12.3.2 The adverse event is not serious, but is **UNEXPECTED** and its association with the study drug, device, or research-related procedure is either **DEFINITELY**, **PROBABLY**, or **POSSIBLY RELATED,** or **UNKNOWN** (as defined above).

Federal policy [45 CFR 46.116(b)(5)] also requires that investigators inform subjects of any important new information that might affect their willingness to continue participating in the research. When an adverse event necessitates changes to the consent/assent form(s) and/or protocol, or that notification is given to currently or previously enrolled subjects, an amendment request will be submitted in conjunction with the adverse event report. The IRB will make a determination whether any new findings, new knowledge, or adverse effects should be communicated to subjects.

In accordance with IRB guidelines, serious adverse events will be reported within 10 days of learning of the event to the Office of Research Risks Protection, Room 300, Research Foundation Building, 1960 Kenny Road, CAMPUS, 614-688-8457 telephone, 688-0366 fax, Email: researchrisksinfo@osu.edu, using the Event Reporting Form (http://orrp.osu.edu/irb/event/documents/EventReportingForm_v2.2.doc) of The Ohio State University Institutional Review Boards. If the adverse event involved the death of a subject, it will be reported immediately, usually within 72 hours. Deaths from "natural causes" or underlying disease that occur more than 30 days following completion of study interventions (i.e., events not temporally associated) need not be reported. Unexpected adverse events that are not serious but may be associated with the drug, device, or procedure (see below) should generally be reported to the IRB within 30 days of notification of the event.
In some instances, adverse events or "unanticipated problems" result in social or psychological harm rather than physical harm to subjects or others. These events should also be reported to the IRB within 30 days, unless they are considered "serious". A letter format may be used for reporting these events instead of the Event Reporting Form, as applicable.

The IRB will review all serious adverse event reports to reevaluate the risks and benefits of the research and need for changes. All other reportable adverse events (unexpected and related or unknown) will be reviewed administratively, unless IRB review is recommended. All investigators will be notified of any action taken, usually within 30 days.

13. CRITERIA FOR RESPONSE ASSESSMENT

All patients who took the intervention for any period of time will be considered evaluable. Participants with only baseline measures and who did not receive the intervention will be considered as drop-outs. RECIST v. 1.1 will be utilized to evaluate responses to therapy.

14 STUDY CALENDAR

	Week				
Tests & observations	**0**	**4**	**8**	**12**	**16**
Signed informed consent	x				
History and Physical Exam	x				x
Height/weight	x	x	x	x	x

Standard of Care Imaging	x		x		x
Continuous Glucose/Ketone Monitoring	x		x		x
Ketone Finger Stick	x	x	x	x	x
Activity Tracker	x	x	x	x	x
DXA Bone & Body Fat Quantification	x				x
Cognitive Testing	x		x		x
Review of medications	x	x		x	
Performance status	x		x		x
SF-36	x			x	
Brief Pain Inventory Questionnaire (BPI-10)	x		x		x
Brief Fatigue Inventory	x		x		x
FACT Questionnaire	x		x		x
Hearth Hope Index	x		x		x
Blood tests	x		x		x
FFQ questionnaire/3-day food log*	x				

15 CRITERIA FOR REMOVAL OF PATIENTS FROM PROTOCOL THERAPY

Study patients may voluntarily withdraw at any time from the protocol. If a treating physician elects to remove a patient from the study, the Principal Investigator must be notified of withdrawal from the protocol. The reasons for discontinuation of the study must be documented in the patient record and data collection forms. Patients experiencing irreversible Grade 3-4 toxicity that is clearly related to the study treatment will be removed from the protocol. Patients with documented progression of disease will be removed from the protocol.

16 ETHICAL AND REGULATORY CONSIDERATIONS

This trial will be conducted in compliance with the protocol, Good Clinical Practice guidelines, and all applicable regulatory requirements.

16. 1 Institutional Review Board

The Principal Investigator will have obtained written approval to conduct the study from The Ohio State University IRB and the Clinical Scientific Review Committee of the James Cancer Hospital and Solove Research Institute. All amendments must be approved by the Institutional Review Board of The Ohio State University prior to implementation.

16.2 Informed consent

All potential candidates for the study will be given a copy to read of the consent form for the study. The Principal Investigator and/or designee will explain all aspects of the study in lay language and answer all the candidate's questions regarding the study. If the candidate desires to participate in the study, she will be asked to sign the Informed Consent. The study agent will not be released to a subject without a signed Informed Consent.

Elements of informed consent include explanations of 1) the purpose of the trial, 2) what the study

entails, 3) alternate treatments, 4) expenses and inconveniences to be incurred, 5) discomfort and risks to the subject, 6) whether she will receive payment for participation in the study, 7) contact person to call in the event of an emergency, 8) subject rights as a result of illness or injury from trial participation, 9) her right to withdraw from the trial at any time without prejudice, 10) confidentiality of trial participation.

16.3 Patient confidentiality

The information obtained during the conduct of this study is considered confidential and will not be released without the written permission of the subject, except as necessary for monitoring by the FDA or other regulatory agencies. All laboratory specimens will be labeled with coded identifiers in order to maintain confidentiality. Signed consent forms, data sheets, and laboratory notebooks will be kept in locked cabinets in Dr. Maryam Lustberg's or Dr. Jeff Volek's office and/or research laboratories.

16.4 Publication of research findings

Publications of the research findings will present data in a format that will not reveal the identity of the participants.

16.5 Compliance monitoring

In accordance with IRB guidelines, the study program will be reviewed by the IRB every 12 months or less. Deviations from the protocol must be documented in the medical record and reported immediately to the PI. Deviations that meet the criteria for Immediate Event Reporting (http://orrp.osu.edu/irb/event/index.cfm) such as those that increase risks to subjects and/or compromise scientific integrity will be reported immediately to the IRB.

16.6 Biosafety

This project will involve the use and analysis of human cells and tissues. Specific precautions will be taken to protect laboratory personnel and support personnel form possible infective agents from these samples, with the goals of containment of biological materials, proper waste disposal, routine decontamination of equipment and surfaces, and implementation of procedures for accidents.

16.7 Data Safety Monitoring Plan

The data and safety monitoring plan will involve the continuous evaluation of safety, data quality and data timeliness. Investigators will conduct continuous review of data and patient safety at their regular Disease Group meetings (at least monthly) and the discussion will be documented in the minutes. The Co-PIs of the trial will review toxicities and responses of the trial where applicable at these disease center meetings and determine if the risk/benefit ratio of the trial changes. Frequency and severity of adverse events will be reviewed by the Co-PIs and compared to what is known about the agent/device from other sources; including published literature, scientific meetings and discussions with the sponsors, to determine if the trial should be terminated before completion. Serious adverse events and responses will also be reviewed by the OSUCCC Data and Safety Monitoring Committee (DSMC). The Co-PIs will also submit a progress report biannually that will be reviewed by the committee per the DSMC plan. All reportable Serious Adverse Events (SAE) will also be reported to the IRB of record as per the policies of the IRB.

17.0 STATISTICAL ANALYSES

Analysis Plan

Unless otherwise specified, all analyses will proceed on an intent-to-treat basis, and hypothesis tests will be two-sided and at the 5% significance level.

The primary objective is to assess the feasibility of maintaining a ketogenic versus AICR diet in patients with brain metastasis selected for radiosurgery. The primary measurements are blood levels of glucose and ketones. Measurements will be taken at baseline, 8 weeks, and 16 weeks. These biomarker outcomes will be analyzed using mixed effects linear regression with fixed effects for timepoint, diet group, their interaction, BMI, and sex. We will use subject-level intercept random effects to account for correlation between repeated measurements within subjects. The primary targets of inference are the treatment effects at each (post-baseline) timepoint. To test the two primary measurements at the two post-baseline timepoints, we will use a 4-way Bonferroni-Holm correction to account for multiplicity. Retention at week 8 and 16, as well as side effects, will be summarized and compared between diet groups.

To assess the preliminary efficacy of ketogenic versus AICR diet on tumor response to targeted radiation therapy, we will use a Chi-squared test for the association between treatment group and partial-or-complete response according to RECIST v1.1.

For the exploratory objective assessing the preliminary effects of the ketogenic versus AICR diet on quality of life in BM individuals undergoing radiation therapy, we will use the same linear mixed effects model as for the primary objective, but with the following quality-of-life endpoints as outcomes: Hearth Hope Index, SF-36, FACT-B, Brief Pain Inventory, and Brief Fatigue Inventory. Tests will be performed without multiplicity correction.

The mixed models used for the primary analyses are applicable to unbalanced data due to chance imbalances, missing data, or drop-out. We will assess the possible impact of non-random missingness using sensitivity analyses.

Power Analysis

We base our power analysis on preliminary data from a single-arm trial involving patients with Stage IV disease undergoing treatment plus a ketogenic diet. The changes in blood glucose from baseline to 3 months have mean 11.25 mg/dL and standard deviation 1.077 mg/dL, and the changes in blood ketone levels have mean 0.6 mmol and standard deviation 0.16 mmol. With 12 completers per arm and a significance level of 0.0125 to account for multiplicity, a two-sample t-test has 99% power to detect differences in mean changes between diet arms of 2.3 mg/dL in glucose levels and 0.34 mmol in ketone levels. We expect our tests based on linear mixed models to have slightly higher power.

17.1 Imaging Response Criteria (for schedule of evaluations see Section X)

The typical standard of care MRI protocol at each evaluation will be scored as follows:

17.1.1 Unresected Brain Metastases treated with radiosurgery

The response score will be rated as one of the following (follow-up MRI will be compared to the prior MRI scans):

Stable disease: Absence of disease progression of treated lesion.

Disease progression:

For lesions measuring **more than 5 mm** in the baseline (volumetric) scan: At least 50% increase in the product of the two largest perpendicular diameters (compared to the smallest product measured for the same lesion).

For lesions measuring **5 mm or less** in the baseline (volumetric) scan: At least 100% increase in the product of the two largest perpendicular diameters (compared to the smallest product measured for the same lesion).

Note: Radionecrosis will not be considered tumor progression.

17.1.2 Resected Brain Metastasis (i.e. surgical cavity)

Note: Tumor bed control is defined as the absence of new nodular contrast enhancement in the surgical bed. By definition a post-operative MRI brain scan is required as a baseline study. If there is *questionable* development of nodular enhancement in the surgical bed, this should be graded as stable disease recognizing follow-up studies will make this determination more certain (e.g. questionable area of nodular enhancement continues to grow and should therefore be coded as disease progression). If on a subsequent scan or on an additional imaging modality it is deemed as progression in the surgical cavity according to the criteria above, the date of progression diagnosis will be documented as the first time a new/progressing lesion was evident on a scan (i.e. backdated).

The response score will be rated as one of the following (follow-up MRI brain scans will be compared to the prior MRI brain scan):

Stable disease: Absence of new nodular contrast enhancement in the surgical bed.

Disease progression: Development of new nodular contrast enhancement in the surgical bed.

Note: Radionecrosis will not be considered tumor progression.

17.1.3 Distant Brain Status (excludes status of surgical cavity and any unresected lesions treated with SRS at time of trial enrollment)

The response score will be rated as one of the following (follow-up MRI brain scans will be compared to the prior MRI brain scan):

Stable disease: Absence of new lesions.

Disease progression: Any new lesion, seen on:

a. Two consecutive axial slices, OR

b. At least one slice in two separate planes.

A lesion is designated "Undetermined" as long as it does not comply with the definition of progression due to a new lesion. If on a subsequent scan or on an additional imaging modality it is deemed as cerebral progression according to the criteria above, the date of progression diagnosis will be documented as the first time a new/progressing lesion was evident on a scan (i.e. backdated).

17.1.4 Leptomeningeal Disease (LMD) Status.

1. The response score will be rated as one of the following (follow-up MRI brain scans will be compared to the prior MRI brain scan). It is recommended that if leptomeningeal disease progression is suspected, MRI imaging of the spine should be performed prior to CSF cytology:

Stable disease: Absence of new leptomeningeal lesions.

Leptomeningeal Disease (LMD) progression: Any new lesion:

a. New enhancement along the cranial nerves with neurologic symptoms

b. New enhancement along the cerebellar folia or pial lining outside of thE surgical bed (treated PTV)

c. Focal or diffuse leptomeningeal enhancement distant from treatment sites

d. CSF cytology positive

e. Leptomeningeal enhancement along the spine

A lesion is designated "Undetermined" as long as it does not comply with the definition of progression due to a new lesion. If on a subsequent scan or on an additional imaging modality it is deemed as leptomeningeal progression according to the criteria above, the date of progression diagnosis will be documented as the first time a new/progressing lesion was evident on a scan (i.e. backdated).

- If there is leptomeningeal disease progression it will be classified as "local" vs. "diffuse" as defined below:

  a) Local LMD: within 3 cm of the surgical bed respecting anatomic boundaries (e.g., tentorium, falx, etc.) with absence of evidence of diffuse LMD

  b) Diffuse LMD: any LMD away from surgical bed and/or positive CSF

- Radionecrosis. Meeting any of the following criteria will qualify as radiation necrosis after prior SRS.

  1. Pathologic diagnosis after either resection or biopsy revealing findings consistent with radiation necrosis with the absence of active tumor cells.

  2. Conventional MR

  a. Lesion quotient of < 0.3, where lesion quotient is defined as the proportional value of the maximum axial cross-sectional area of the T2-weighted defined lesion over the maximum axial cross-sectional area of the contrast-enhancing lesion on the T1-weighted post-gadolinium sequence on a comparable axial slice OR

  b. Due to concerns of radiation necrosis, steroids initiated and follow-up scan (>1 month interval on repeat scan) reveals decrease in both edema and contrast of enhancing lesions that were concerning for radiation necrosis.

  3. Centers that normally use PET, MR Perfusion, or MRS to determine a diagnosis of radionecrosis are permitted to use these modalities to diagnose radionecrosis as according to the local PI's best practice.

NOTE: Images for standard restaging time points should continue to use the same imaging modality as used at baseline.

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