Glutamine for the Prevention of Radiation Toxicity in Subjects Undergoing Breast Conserving Therapy

A Phase II Pilot Study
IRB #202253

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1. *ABBREVIATIONS*

AE- adverse event
ANOVA - Analysis of variance
APBI - accelerated partial breast irradiation
ASTRO - American Society of Therapeutic Radiation Oncology
BCT - Breast conserving therapy
C*3*PR - *cancer Biomedical Informatics Grid (caBIG®, NCI) application*
CCTO – Cancer Clinical Trials Office
cGY - centigray
cm - centimeter
CRA – clinical research associate
CRF – case report form
DCIS – Ductal carcinoma in situ
DNA - Deoxyribonucleic acid
FDA – Food and Drug Administration
g – gram
GCP – good clinical practice
GLM – Glutamine
GSH – Glutathione
HSP –heat shock proteins
ICH – International Conference on Harmonization
IRB – Institutional Review Board
kcal – kilocolarie
kg – kilogram
LENT/SOMA – Late Effects Normal Tissue Task Force subjective, objective, management and analytic
MSG – monosodium glutamate
NSABP - National Surgical Adjuvant Breast and Bowel Project
NK – natural killer
PI – principal investigator
PRMC – Protocol Review and Monitoring Committee
RNA - Ribonucleic acid
ROS – reactive oxygen species
RTOG - Radiation Therapy Oncology Group
SAE – serious adverse event
SD – standard deviation
TID – three times a day
UAMS - University of Arkansas for Medical Sciences
UPIRTSO/UPR – unanticipated problems involving risk to subjects or others
XRT - Radiation therapy

2. PROTOCOL SUMMARY

Primary Objective

To determine if oral glutamine (GLN) will reduce radiation toxicity for the subjects undergoing breast conserving therapy (BCT)

Aim 1

To evaluate GLN as an agent to prevent acute and late radiation toxicities and improve the quality of life for the subjects undergoing BCT

Aim 2

To elucidate the mechanisms of GLN effects by examining the antioxidant defense and immune response in host

Study Population

Subjects with DCIS, or Stage I or II Invasive Breast Cancer who are scheduled to undergo accelerated partial breast irradiation as part of BCT

Inclusion Criteria

- Subject have been diagnosed with DCIS, or Stage I or II invasive breast cancer
- Subject will undergo APBI as part of BCT
- Subject is eligible for APBI based on ASTRO (American Society of Therapeutic Radiation Oncology) criteria
- Subject is 18 years of age or older
- Subject is female

Exclusion Criteria

- History of uncontrolled, clinically significant lung, heart, endocrine, liver, or renal disease
- Subject has been diagnosed with any other cancer within 5 years
- Subject has a known hypersensitivity reaction to the following: GLN, glutamate, MSG (i.e., Chinese restaurant syndrome)
- Subject has history of collagen vascular disease
- Subject has uncontrolled Diabetes mellitus I or II
- Subject has had any prior breast radiation
- Subject is pregnant or breastfeeding

Investigational Product

Glutamine/Placebo

Study Design

This Phase II pilot study is a single-centered, double-blind, two-arm randomized study to determine if oral GLN will reduce radiation toxicity for the subjects undergoing BCT. Sixty females with DCIS, or Stage I or II invasive breast cancer will be recruited from the University of Arkansas for Medical Sciences (UAMS) to ensure that 50 complete the study.

3. BACKGROUND

Breast conserving therapy (BCT), which includes lumpectomy and postoperative radiation therapy (XRT), is accepted standard of care for DCIS, and Stage I and II invasive breast cancer. While the efficacy of such an approach has been demonstrated, recent studies have focused on altering radiotherapy techniques to improve local tumor control rate, decrease time of treatment and thus completion of therapy and/or decrease morbidity.[1,2] At UAMS, the standard radiotherapy technique is to administer external beam radiation therapy to the lumpectomy cavity plus 2.5cm margin using megavoltage photons to a dose of 3850 cGy in 10 fractions delivered twice daily, with at least 6 hours between treatments for 5 consecutive days. However, all radiotherapy techniques induce toxicities that affect quality of life and can limit the delivery of adequate dose or completion of full dose. Therefore, there is a real need for agents that can protect normal host tissues while not affecting or even increasing tumor cell damage.

Radiotherapy produces ions and/or free radicals in its passage through the tissue, causing immediate chemical alterations in biological tissues including normal tissue and cancerous tissue.[3] It is thought that radiation-induced side effects are caused, in part, by chronic oxidative stress and inflammation. The produced reactive oxygen species (ROS) either directly interact with the target molecules such as DNA, lipids, etc., or indirectly to form the active elements through various chemical interactions, leading to lipid peroxidation, oxidation of DNA and proteins, as well as activation of pro-inflammatory factors.[4] For example, Delanian et al found that the radiation-induced fibroatrophic process was induced by ROS and mediated by transforming growth factor $\beta 1$ (TGF-$\beta 1$), which could be greatly reduced or reversed via the antioxidant pathway.[5] Therefore, substances with anti-inflammatory and antioxidant effects have long been considered good candidates for protection against radiation toxicity. On the other hand, toxicities and recurrences after radiotherapy may be, in part, the result of immunologic deficits in the host. One study showed the numbers and functions of cells from both the innate and adaptive immune system, including natural killer (NK) cell activity, phagocytic activity of monocytes, and cytokine (TNF-α), were persistently and significantly decreased after the completion of radiotherapy for breast cancer, which were proposed to have consequences for immune response to residual or recurrent malignancy.[6] Another study showed that immune recovery following adjuvant therapy for breast cancer (including radiotherapy) was significantly delayed up to 12 months in various immune parameters, including NK cell and lymphokine-activated killer cell activities, lymphocyte proliferation, cytokine productions (IFN-γ, IL-2, IL-4, IL-6, and IL-1α), and CD cell subsets (CD4, CD8, and CD56), which were thought to contribute to the poor clinical outcomes affecting the quality of life.[7] Therefore, interventions to facilitate a timely recovery of immune responses will potentially benefit patients to improve clinical outcomes.

Our extensive glutamine (GLN) studies and studies performed by other investigators have strongly reinforced that GLN is a candidate for preventing radiation toxicities and recurrences in breast cancer patients undergoing BCT by providing host natural antioxidant and stimulating host immune system, which reverse the mechanisms of radiation toxicities. Treatment with oral GLN has been shown in multiple animal models to significantly reduce both acute and chronic radiation injuries, protect the host, and decrease tumor growth.[8] Oral GLN treatment protected the intestinal mucosa and accelerated healing of the small intestine after radiation injury and prevented chronic radiation enteropathy.[9,10] It has also been shown to replenish and elevate host GLN stores and support muscle GLN metabolism without stimulating tumor growth in a rat model.[11] Oral GLN also decreased tumor growth by enhancing NK cell activity in the tumor-bearing rat model.[12] GLN also prevented chemical 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary tumors through enhancing glutathione (GSH) levels in normal tissues (three fold in breast tissue), while reducing GSH levels in tumor cells, effectively sensitizing the tumor to radiation.[13] Recently Kuhn et. al. reviewed the evidence from both experimental and clinical studies of glutamine supplementation, confirming an improvement in host metabolism without increasing tumor growth. There is evidence for an enhancement of the tumoricidal effect of radiotherapy by GLN, in addition to a reduction in toxicity to host tissues through increasing GSH and modulation of apoptotic regulators and host immune response, and induction of heat shock proteins (HSPs).[14] Furthermore, following tissue injury, the wound healing response

relies upon an efficient function of fibroblasts, which require GLN as a rate-limiting amino acid for their growth. GLN promotes DNA synthesis and proliferation of cultured fibroblasts and plays a central role in the metabolism of rapidly dividing cells by providing the nitrogen for the synthesis of nitrogenous compounds used in the manufacture of DNA, RNA and other macromolecules.[15] Thus, the increased availability of GLN in the microenvironment following GLN administration may lead to more efficient wound healing. On the other hand, wound healing and repair is critically regulated by ROS. If ROS are produced in excessive amount, oxidative stress occurs, resulting in severe cell damage. GLN treatment may also aid in wound healing and repair through increasing host antioxidant GSH.[16]

4. TRIAL OBJECTIVES AND AIMS

a. Primary Objective
To determine if oral GLN will reduce radiation toxicity for subjects undergoing BCT.

b. Aim 1
Evaluate GLN as an agent to prevent acute and late radiation toxicities and improve the quality of life for subjects undergoing BCT.
We will investigate oral GLN treatment in a cohort of 50 subjects diagnosed with DCIS, or Stage I or II invasive breast cancer, undergoing accelerated partial breast irradiation (APBI) as part of BCT. Subjects will be randomized in a 1:1 ratio to receive GLN versus placebo and followed daily during the treatment with APBI and then at 1, 6 and 12 months after APBI to determine differences in clinically evident acute and late radiation toxicity through multiple assessments. The primary efficacy outcome will be excessive toxicity, which will be defined as a score of 2 or higher using the RTOG Acute scale of radiation-toxicity criteria when scored on either the 12-day or 30-day assessment time.

c. Aim 2
Elucidate the mechanisms of GLN effects by examining the antioxidant defense and immune response in host.
To examine antioxidant defense, we will measure non-enzymatic and enzymatic antioxidants, reactive oxygen metabolites, lipid peroxidation, oxidative DNA damage, and protein oxidation in subjects treated with GLN versus placebo. We will also test a potential biomarker for the GLN effect. To evaluate the immune response, we will measure complete blood count, NK cell activity, and cytokine production in these subjects.

5. SUBJECT POPULATION

Eligibility Criteria
Subjects are eligible for the study if the following inclusion and exclusion criteria are met:

Inclusion Criteria
- Diagnosed with DCIS, or Stage I or II invasive breast cancer
- Subject will undergo APBI as part of BCT
- Eligible for APBI based on ASTRO (American Society of Therapeutic Radiation Oncology) criteria
- Female and 18 years or older

Exclusion Criteria
- Subject has history of uncontrolled, clinically significant lung, heart, endocrine, liver, or renal disease

- Subject has been diagnosed with any other cancer within 5 years
- Subject has any type of hypersensitivity reaction following exposure to GLN, and/or glutamate, and/or MSG (i.e., Chinese restaurant syndrome)
- Subject has a history of collagen vascular disease
- Subject has been uncontrolled Diabetes mellitus I or II
- Subject has had any prior breast radiation
- Subject is pregnant or breastfeeding

Accrual Goal

Up to 60 subjects will be enrolled to ensure 50 subjects complete the study.

Recruitment Plan

Patients with DCIS, or Stage I or II Invasive Breast Cancer that are scheduled to undergo accelerated partial breast irradiation as part of Breast conserving therapy will be identified in the UAMS Breast Surgical Oncology clinic during routine clinic appointments by their providers. Upon identification, the patient will be informed of the study by their physician along with the research staff.

6. INVESTIGATIONAL DRUG - Glutamine/Placebo

a. General Description

Glutamine is a non-essential amino acid and is available as a white crystalline powder, which is soluble in water. Dextrose monohydrate is a form of sugar that is available as a white, crystalline or granular powder and is freely soluble in water.

b. Manufacturing and Formulation

Glutamine will be ordered from a commercially available supplier. Glutamine's molecular formula is: $C_5H_{10}N_2O_3$ and molecular weight is 146.15 g/mol.

c. Preparation

Glutamine and Dextrose powders will be re-packaged into opaque jars, along with disposable measuring scoops for this study. Re-packaging and re-labeling will be done by the UAMS Research Pharmacy staff.

d. Dosing Administration or Utilization

Based on randomization assignment, subjects will be instructed to use measuring scoop to measure out each dose and then mix it with 8 ounces of water. Dosing will be repeated three times a day.

Study drug supply (Glutamine/Placebo) will be dispensed on Day 0 to each study subject.

e. Storage and Disposition

Glutamine and Dextrose monohydrate powder (placebo) will be stored securely in the UAMS Research Pharmacy at room temperature.

f. f. Agent Ordering

Ordering of bulk test article and placebo will be performed by the UAMS Research Pharmacy staff.

g. Agent Accountability

Accountability records will be maintained on this study by the UAMS Research Pharmacy staff. A standardized NCI accountability log or equivalent will be used.

7. TREATMENT PLAN

a. On-Study Evaluation

After signing the IRB-approved informed consent form, research participants will be registered by a member of the research team.

b. Dose Assignment

This is a randomized, doubly blinded, placebo-controlled study. Subjects will be randomized to one of two groups; group 1 will receive powdered dextrose (i.e., placebo) while group 2 will receive powdered glutamine. Glutamine and placebo (dextrose) will be stocked in the UAMS Research Pharmacy. Arrangements will be made with the pharmacist to dispense the medications in dosages to be glutamine (30 g/day – 10 g three times a day (TID)), dextrose (25 g/day). Daily doses will be divided into a TID administration. If any doses are missed they can be added to the next scheduled dose. The powder may be mixed with any cold liquid. Medication will be dispensed in bulk and a measuring scoop appropriate for the subject's dose will be provided. Participants will be given a 30 day supply of medication. Dextrose was chosen as a control as it looks like the glutamine powder to be administered. This amount of Dextrose (approximately 100 kcal) is equal to one Hershey kiss three times a day so a very small dose of carbohydrate calories.

Subject eligibility will be established before treatment randomization. Once a subject's eligibility is determined, the UAMS Research Pharmacist will be notified to implement the randomization process. Randomization will be completed when Pharmacy receives a copy of signed consent and physician's orders for study agent to be dispensed.

Block randomization will be done using randomly chosen block sizes of 2, 4, and 6 to maximize the likelihood that treatment arms will have equal numbers of patients. Randomization will be done by sealed envelopes located at the UAMS Research Pharmacy. There will be one set of consecutively numbered envelopes. At each randomization, the lowest numbered envelope in the set will be chosen. The Pharmacist will open the envelope and assign the subject to the treatment arm based on the contents of the envelope.

Based on the results of the randomization, the appropriate treatment will be dispensed to the subject. A list of randomized subjects will be kept confidential and securely stored in the UAMS Research Pharmacy.

- Methods for ensuring blinding

Only the UAMS Research Pharmacy staff will be unblinded. The blinded drugs and the blinded code will be stored in a safe and secure location in the UAMS Research Pharmacy.

- Methods for unblinding the study

Upon completion of the study: The drug codes will be broken and made available to the Statistician, the Principal Investigator and the Co-investigator for final data analysis.

PROCEDURE FOR UNBLINDING IN CASE OF MEDICAL EMERGENCY: In the event of medical emergency, the treating physician will contact the UAMS Research Pharmacy to unblind the patient's treatment assignment. A subject's treatment assignment should only be unblinded when knowledge of the treatment is essential for further management of the subject. Unblinding for any other reason will be considered a protocol deviation. Unblinding must be documented in the subject's CRF.

Arms	Assigned interventions
Placebo – Group 1	Powdered Dextrose, 8.33 grams by mouth TID for 30 days, so that daily dose is 25 grams per day.
Glutamine – Group 2	Powdered Glutamine, 10.0 grams by mouth TID for 30 days, so that daily dose is 30 grams per day. .

c. Specimen Handling

Blood (approximately 45ml) will be collected in Sodium Heparin (green top) tubes and 5 ml blood collected in serum (red top) tubes. Samples will be collected in the Cancer Institute Blood Draw station or the Infusion room by a trained personal. Tubes will be labeled with each subject's unique identification number or code. Tubes will be delivered to Dr. Kaufmann's laboratory for analysis.

d. Visit Breakdown

Baseline Visit:

- The subject will sign the consent form.
- A quality of life questionnaire will be completed by the subject.
- Performance status will also be determined.
- Subject will have breast skin graded by RTOG acute scoring system.
- Subject and physician point scoring system for breast cosmesis will be completed.
- Blood will be drawn for the measurements of non-enzymatic and enzymatic antioxidants, reactive oxygen metabolites, lipid peroxidation, oxidative DNA damage, protein oxidation, myoglobin, complete blood count, NK cell activity, and cytokine production. Blood (approximately 45ml) will be collected in Sodium Heparin (green top) tubes and 5 ml blood collected in serum (red top) tubes. A bilateral digital mammogram will be performed in the range $\leq$ 6 months prior to lumpectomy as per standard treatment and density will be determined.
- Subject will obtain supply of medication from the outpatient pharmacy after subject is randomized by the pharmacist.
- Administration of the GLN/placebo will begin at Day 1 with the instructions for taking the initial dosage of the oral medication given to the subject. The subject will remain on medication from this point; continue through APBI, and for 2 weeks following completion of APBI. GLN/placebo will be discontinued at the Month 1 visit.
- A Glutamine diary will be given to the subject with instructions. Subjects are to record daily intake of Glutamine along with any events they may experience that the believe maybe related to Glutamine.

- The next appointment for the subject will be made and subject will be released from either the UAMS Breast Surgical Oncology clinic or the UAMS Radiation Oncology Center.

Day 8 Visit:

- The subject will begin APBI as part of routine care.
- Blood will be drawn for the measurements of non-enzymatic and enzymatic antioxidants, reactive oxygen metabolites, lipid peroxidation, oxidative DNA damage, protein oxidation, myoglobin, complete blood count, NK cell activity, and cytokine production. Blood (approximately 45ml) will be collected in Sodium Heparin (green top) tubes and 5 ml blood collected in serum (red top) tubes.
- The next appointment for the subject will be made and subject will be released from the UAMS Radiation Oncology Center.

Day 9 Visit:

- Adverse Event assessment
- The next appointment for the subject will be made and subject will be released from the UAMS Radiation Oncology Center.

Day 12 Visit:

- Blood will be drawn for the measurements of non-enzymatic and enzymatic antioxidants, reactive oxygen metabolites, lipid peroxidation, oxidative DNA damage, protein oxidation, myoglobin, complete blood count, NK cell activity, and cytokine production. Blood (approximately 45ml) will be collected in Sodium Heparin (green top) tubes and 5 ml blood collected in serum (red top) tubes.
- A quality of life questionnaire will be completed by the subject.
- Performance status will also be determined.
- The subject will have breast skin graded by RTOG acute scoring system.
- A subject and physician point scoring system for breast cosmesis will be completed.
- The next appointment for the subject will be made and subject will be released from the UAMS Radiation Oncology Center.
- Adverse Event assessment

Month 1 Visit:

- The subject will complete GLN/placebo.
- Subject will return any unused GLN/placebo to monitor treatment compliance (measure unconsumed product). This will be used to measure compliance as a percentage of product dose taken. It will also be used to correlate with serum levels of glutamine. Analysis will be performed on intent to treat.
- Glutamine diary will be returned to the study personnel.
- Blood will be drawn for the measurements of non-enzymatic and enzymatic antioxidants, reactive oxygen metabolites, lipid peroxidation, oxidative DNA damage, protein oxidation, complete blood count, NK cell activity, and cytokine production. Blood (approximately 45ml) will be collected in Sodium Heparin (green top) tubes and 5 ml blood collected in serum (red top) tubes.
- A quality of life questionnaire will be completed by the subject.
- Performance status will also be determined.
- The subject will have breast skin graded by RTOG acute scoring system.
- A subject and physician point scoring system for breast cosmesis will be completed.
- An open-ended question for subjects regarding their experience with the study supplement (glutamine/placebo) and radiation treatment will be completed by the subjects.
- Subjects will be examined for recurrence.
- Adverse Event assessment
- The next appointment for the subject will be made and subject will be released from the either the UAMS Breast Surgical Oncology clinic or the UAMS Radiation Oncology Center.

Month 6 Visit:

- A bilateral mammogram will be performed.
- The subject will continue to be grade by per guidelines on RTOG LENT/SOMA Scoring System.
- A quality of life questionnaire will be completed by the subject.
- Performance status will also be determined.
- An open-ended question for subjects regarding their experience with the study supplement (glutamine/placebo) and radiation treatment will be completed by the subjects.
- Subjects will be examined for recurrence.
- A subject and physician point scoring system for breast cosmesis will be completed.
- Adverse Event assessment
- The next appointment for the subject will be made and subject will be released from the either the UAMS Breast Surgical Oncology clinic or the UAMS Radiation Oncology Center.

Month 12 Visit:

- A bilateral mammogram will be performed.
- The subject will be grade by per guidelines on RTOG LENT/SOMA Scoring System.
- Subjects will be examined for recurrence.
- A subject and physician point scoring system for breast cosmesis will be completed.
- Adverse Event assessment

e. Prohibited Medications

Lactulose

f. Dose Limiting Criteria

None

8. STUDY CALENDAR

	Base line[e]	Day 1 to Day 7	Day 8[b]	Day 9	Day 10	Day 11	Day 12[c]	Day 13 to Day 30	Month 1 ±2wk	Month 6 ±1mo	Month 12 ±1mo
Informed Consent	x										
GLN		x	x	x	x	x	x	x			
APBI			x	x	x	x	x				
RTOG Acute	x						x		x		
Cosmetic score	x						x		x	x	x
RTOG Chronic										x	x
CBC	x		x				x		x		
Myoglobin	x		x				x				
Study Lab[d]	x		x				x		x		
Mammogram/Breast Density[a]	x									X	X
Performance Status	x						x		x	x	
A quality of life questionnaire	x						x		x	x	
An open-ended question									x	x	
Adverse event assessment				x			x		x	x	x

a. A bilateral digital mammogram will be performed in the range ≤ 6 months prior to lumpectomy as per standard treatment to rule out recurrence and measure density.
b. Day 8 is start of APBI.
c. Day 12 is end of APBI.
d. Blood will be drawn for the measurements of non-enzymatic and enzymatic antioxidants, reactive oxygen metabolites, lipid peroxidation, oxidative DNA damage, protein oxidation, myoglobin, complete blood count, NK cell activity, and cytokine production.
e. A window of 2 weeks will be allowed from Baseline to Day 1. All baseline procedures must be completed prior to subject starting glutamine.

9. RISKS AND TOXICITIES TO BE MONITORED

a. Potential Toxicities, Risks and Precautions

Procedure	Risks	Measures to Minimize Risks
Complete history and physical exam, including blood chemistries	Identification of previously unknown condition	Qualified health care provider to evaluate potential subject
Collection of blood samples	Pain, bruising at the injection site and rarely infection Discovery of previously unknown conditions Possible breach of confidentiality	Experienced personnel will perform the phlebotomies using approved techniques. Pressure and dressings will be used to minimize pain, bruising and infection. Research records are kept in a locked area accessible only by study personnel.

Procedure	Risks	Measures to Minimize Risks
		Subject study numbers will be used for identification of samples so that confidentiality is ensured.
Glutamate sensitivity	Headache, flushing, sweating, facial pressure or tightness, numbness, tingling or burning in the face, neck or other areas, rapid, fluttering heartbeats, chest pain, nausea, and/or weakness	Qualified health care provider to evaluate potential subject
Dextrose sensitivity	High Blood sugar: (Tissue damage, coma,death)	Subjects with uncontrolled Diabetes mellitus I or II are excluded from the study Qualified health care provider to evaluate potential subject
Collection of data	Possible breach of confidentiality	Research records are kept in a locked area accessible only by study personnel. Subjects will only be identified by study numbers on all research documents. Investigators will provide certification of completion of human subject protection training course. UAMS shall retain the study documents on file until three years after the completion and final study report of this investigational study.

b. Benefits

There is no guarantee that subjects will receive direct benefit from participation in the study. Subjects may experience a significant decrease in the severity of the effects of radiation therapy to the breast, thereby increasing the general well-being during therapy and aiding to maintain a generally increased quality of life while receiving radiation therapy. Potential additional benefits include decreased chance of long term radiation-induced skin damage, breast scarring and therefore improved cosmetic results.

For subjects assigned to the arm of this study who are receiving dextrose, no benefit will be anticipated.

c. Injury

There are no foreseeable risks associated with the administration of GLN or dextrose.

10. DATA HANDLING AND RECORD KEEPING

a. Registration Procedures

Subjects will be registered in C3PR, a cancer Biomedical Informatics Grid (caBIG®, NCI) application.

b. Methods for Data Collection and Data Collection Tools

Data will be entered into OpenClinica through electronic web-based case report forms (CRFs) which replicate the paper CRFs attached to this protocol. OpenClinica is a secure open source system for electronic data capture and clinical data management. Data will be stored as required per regulations.

c. Confidentiality, Storage and De-Identification of Data

Government agency regulations and directives require that the study investigator retain all study documentation pertaining to the conduct of a clinical trial. Study documents should be kept on file until three years after the completion and final study report of this investigational study.

11. CRITERIA FOR EVALUATION

a. Outcome measures for Specific Aim 1

i. Primary efficacy outcome:

- Excessive Toxicity, which will be defined as an acute radiation-toxicity score of 2 or higher on either Day 12 (the last day of APBI) or at the one-month follow-up visit. Acute radiation toxicity will be scored as described below using the RTOG Acute toxicity-scoring criteria.

ii. Secondary efficacy outcomes:

- Acute radiation toxicity, which will be scored using the RTOG Acute toxicity-scoring criteria. These range from 0 (no change) to 4 (necrosis). Acute radiation toxicities will be scored on Day 0 (baseline, a week before APBI), Day 12 (the last day of APBI), and at the one-month follow-up visit.
    - A score of 2 or higher on the RTOG Acute scale will be considered to be excessive toxicity if it is scored on either Day 12 or at the one-month follow-up visit (see Excessive Toxicity above).

- Chronic radiation toxicity, which will be scored using RTOG LENT/SOMA criteria. These range from Grade 1 (minimal hypersensation) to Grade 4 (refractory). Chronic radiation toxicities will not be scored at baseline, but will be scored during the follow-up visits at 6, and 12 months.

- Breast cosmesis, which will be scored using a subject-and-physician point-scoring system. Breast cosmesis will be scored at Day 0 (baseline), Day 12 (the last day of APBI), and during the 1 month, 6 month, and 12 month follow-up visit.

- Breast density, which will be determined by mammogram at baseline and during the follow-up visits at 6 and 12 months.

- Performance Status, which will be assessed using questionnaire. Performance status will be assessed on Study Days 0, 12, and during the follow-up visits at 1 and 6 months.

- Quality of life, which will be determined using questionnaire. The quality-of-life questionnaire will be assessed on Study Days 0, 12, and during the follow-up visits at 1 and 6 months.

- An open-ended question (How was your experience with this treatment?), which will be answered by the subject. An open-ended question will be assessed during the follow-up visits at 1 and 6 months.

- Additional subject data to be collected will be BMI, meds, steroids, smoking, alcohol, etc. Stratification using these criteria will be considered but unlikely to be significant in this small pilot trial.

b. Outcome measures for Specific Aim 2

Assays of all outcome measures will be performed on blood samples obtained at the following times from each subject: Day 0 (baseline, the day before GLN/placebo begins), Day 8 (just before APBI commences), Day 10 (the third day of APBI), Day 12 (the last day of APBI), and at the 1-month follow-up visit (the day when GLN/placebo ends).

- Measures of oxidative stress, antioxidant defense and a potential biomarker for GLN effect:
    - Levels of enzymatic antioxidants such as glutathione peroxidase (GPx), glutathione S-transferase (GST), superoxide dismutase (SOD), and catalase (CAT).
    - Levels of non-enzymatic antioxidants, namely, glutathione in both its reduced (GSH) and oxidized (GSSG) form.
    - Reactive oxygen species such as the superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2).
    - Lipid peroxidation products such as malondialdehyde (MDA) and/or 4-hydroxynonenal (4-HNE).
    - DNA-oxidation products such as 8-hydroxy-2'-deoxyguanosine (8-OHdG).
    - Protein-oxidation markers such as the carbonyl derivatives of proline, lysine, arginine and threonine residues.
    - A potential biomarker for GLN effect such as myoglobin.

- Measures of immune response:
    - Complete blood count (CBC)
    - NK-cell activity.
    - Production of cytokines such as interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α).

12. STATISTICAL CONSIDERATIONS

a. Sample Size and Power

Sample-size considerations are motivated by the statistical analysis plan for the primary efficacy outcome (see below), in which a two-sided Fisher's exact test will be used at 10% alpha to compare the glutamine arm to the placebo arm for a statistically significant decrease in the proportion of excessive toxicities. Rubio *et al.*[17] reported the results of a pilot study of oral glutamine supplementation versus placebo in 17 breast-cancer subjects receiving radiation as part of breast-conservation therapy. By the 7th week of their study, all 8 subjects taking placebo (100%), but only 4 of 9 subjects taking glutamine (44%), experienced scores of ≥2 on the RTOG Acute toxicity scale. Their results are consistent with excessive-toxicity rates of at least 90% on the placebo arm, but at most only 50% on the glutamine arm. To calculate power, we hypothesize that excessive toxicity rates in our study will be equal to 50% with glutamine versus 90% with placebo. Calculations using PASS 12 software[18] show that a total of 50 study subjects, 25 randomized to the glutamine arm and 25 randomized to the placebo arm, give the two-sided Fisher's exact test 91.1% power at 10% alpha to detect the hypothesized difference in excessive-toxicity rates between the glutamine arm and the placebo arm. This result demonstrates that 25 subjects per study arm (50 subjects total) give this proposed study sufficient

power to achieve the radiation-toxicity-reduction part of its primary objective. To protect against dropouts, a total of 60 subjects will be enrolled and randomized in order to ensure that 50 subjects complete the study.

b. Statistical analysis plan for Specific Aim 1

i. Analysis plan for the primary efficacy outcome

The number of excessive toxicities on each study arm will be reported both as a raw count and as a proportion of the number enrolled per arm. The study-arm difference in excessive-toxicity proportions will be tested for statistical significance using a two-sided Fisher's exact test at an alpha=10% significance level.

ii. Analysis plan for the secondary efficacy outcomes

Data for each secondary-efficacy outcome measure will be summarized by assessment time and study arm, using mean scores and proportions for Likert-scaled data (acute radiation toxicity, chronic radiation toxicity, and performance status), and using means and standard deviations (SDs) for continuous and quasi-continuous data (, breast cosmesis, and quality of lifeData will be graphed versus assessment time as stacked barcharts or profile plots depending on the outcome measure. Treatment-arm differences over time will be assessed using longitudinal-analysis methods, namely, (A) logistic and cumulative-logistic regressions with generalized estimating equations for dichotomized and Likert-scaled data, respectively, and (B) repeated-measures ANOVAs for continuous and quasi-continuous data. Post-hoc comparisons between study arms at each time point will employ an alpha=5% significance level despite the multiple testing, in order not to inflate Type II error in this pilot Phase II study.

c. Statistical analysis plan for Specific Aim 2

For each assay endpoint, data will be summarized by study arm and collection timepoint as means and SDs, and graphed versus time as profile plots. Adherence to distributional assumptions will be examined, and data transformations will be applied if warranted. Repeated-measures ANOVA will be used to compare each assay endpoint for study-arm differences over time. Post-hoc comparisons between study arms at each time point will employ an alpha=5% significance level despite the multiple testing, in order not to inflate Type II error in this pilot Phase II study.

d. Missing, Unused and Spurious Data

Missing data will be treated as missing, and will not be imputed. Spurious data will be corrected at the source document. Any data documented as spurious that is unable to be corrected at the source will be treated as missing.

13. ETHICAL AND REGULATORY CONSIDERATIONS

The following must be observed to comply with FDA regulations for the conduct and monitoring of clinical investigations. The following also represents sound research practice. All study personnel must have completed training in good clinical practice (GCP) and protection of human subjects.

a. Recruitment and Informed Consent

Patients with DCIS, or Stage I or II Invasive Breast Cancer that are scheduled to undergo accelerated partial breast irradiation as part of Breast conserving therapy will be identified in the UAMS Breast Surgical Oncology clinic during routine clinic appointments by their providers. Upon identification, the patient will be informed

of the study by their physician along with the research staff. The patient will be allowed to review the IRB-approved informed consent form, and if they would like to participate in the study, they will be presented the informed consent form. During the consent process, the patient will be clearly informed of the fact that participation in this study is voluntary and that the decision to not participate in the study will have no effect on future clinical care. The patient will be encouraged to have family or friends participate in any or all of the process. The patient will be provided sufficient time to ask questions, and will be questioned to be ensure they understand the information. If the patient agrees to proceed, they will sign consent. The consent process will be documented in the medical record. A copy of the informed consent document will be given to the research participant, and additional copies will be sent to the medical records department. The original informed consent will be filed with the subject file in CCTO. The principles of informed consent are described by the Federal Regulatory Guidelines: Code of Federal Regulations (*21CFR50*) and the Office for Human Research Protections: Protection of Human Subjects (*45CFR46*). These principles must be followed to comply with Food and Drug Administration (FDA) regulations for the conduct and monitoring of clinical investigations.

b. Institutional Review:

This study will be approved by the UAMS Institutional Review Board (IRB) as defined by Federal Regulatory Guidelines *21CFR56* and the Office for Human Research Protections: Protection of Human Subjects *45CFR46*. This study will also undergo scientific review by the Cancer Institute's Protocol Review and Monitoring Committee (PRMC). Approval by both the IRB and PRMC is required before the clinical trial can be activated.

14. ADVERSE EVENTS

a. Expedited Reporting

- The Principal Investigator (PI) must be notified within 24 hours of learning of any serious adverse events (SAEs), regardless of attribution, occurring during the study.
- The UAMS IRB must be notified within 10 business days of "any unanticipated problem involving risk to subject or others (UPR/UPIRTSO)."
- For UPR/UPIRTSO, see UAMS IRB Policy 10.2.

b. Routine Reporting

All other AEs, such as those that are expected, or are unlikely or definitely not related to the study participation, are to be reported annually as part of regular data submission.

15. MONITORING

a. Data Monitor

The PI will monitor the study to ensure that the rights and well-being of human subjects are protected, that the data are accurate, complete and verifiable from source documents and that the trial is conducted in compliance with currently approved protocol/amendments.

16. BIBLIOGRAPHY

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