Title: Effects of Riluzole on CNS Glutamate and Fatigue in Breast Cancer Survivors with High Inflammation

Principle Investigator: Andrew H. Miller MD

Co-Investigators: Ebrahim Haroon MD, Xiaoping Hu PhD, Mylin A. Torres MD, Michael T. Treadway PhD, Bobbi J. Woolwine MSW, Jennifer C. Felger PhD, Brian Dias PhD, Tanja Jovanovic PhD, Seth Norrholm PhD, Vasiliki Michopoulos PhD MS, Wendy Baer, MD, Kelly M. Sklare

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2. Précis/Abstract:

The goal of the proposed research is to determine whether riluzole, a drug that increases glutamate reuptake, will decrease CNS glutamate in breast cancer survivors with increased inflammation and fatigue. We will also determine whether decreasing glutamate with riluzole will reverse inflammation-related fatigue and other symptoms including cognitive dysfunction and decreased motivation. To accomplish these goals, we plan to conduct an 8 week, double-blind, randomized control trial of riluzole (100 mg/d) versus placebo in 40 breast cancer survivors (n=20 per group). All breast cancer survivors will have completed treatment within 1-5 years and have a fatigue level of ≥4 (on a 10 point scale) and a plasma c-reactive protein (CRP) concentration >3mg/L (indicative of high inflammation). Patients will undergo magnetic resonance spectroscopy (MRS) to measure CNS glutamate before and after 1 and 8 weeks of riluzole or placebo treatment. Fatigue and other behavioral assessments including measures of cognitive function, motivation, and startle reactivity will be conducted before and after treatment and correlated with the change in CNS glutamate.

3. Introduction and Background:

Breast cancer is one of the most common cancers among women with ~250,000 new cases diagnosed in the US each year. Significant advances have been made in treating breast cancer, and the current number of women in the US who are considered breast cancer survivors is over 2 million. Despite these advances, breast cancer and its treatment comes at a considerable cost for a significant percentage of women with up to 30% of women experiencing behavioral and/or cognitive symptoms months to years after treatment completion.[1-4] The mechanisms which contribute to these symptoms are only beginning to be understood, however, mounting data suggest that inflammation may be involved.

Work from our group and others have demonstrated significant relationships between inflammatory markers and inflammatory signaling pathways and symptoms of fatigue and cognitive dysfunction in patients with multiple forms of cancer including breast cancer 2,7-11.[2, 5-8] In addition, polymorphisms in genes for inflammatory cytokines have been associated with cancer-related fatigue in breast cancer patients.[9, 10] Moreover, blockade of the inflammatory cytokine TNF-alpha was found to reduce symptoms of fatigue in patients with advanced cancer, suggesting a cause and effect relationship between inflammation and behavioral change.[11]

There are a number of theories as to how inflammation may influence fatigue and cognition function in breast cancer patients. One neurotransmitter pathway that may be involved is glutamate.[12, 13] Inflammatory cytokines have been shown to decrease glutamate reuptake and increase glutamate release from astrocytes.[14-16] Excess glutamate has been shown to

have a neurotoxic effect on neurons through direct excitotoxicity and inhibition of neuronal growth factors such as brain derived neurotrophic factor. [12, 17] In addition to direct effects of inflammatory cytokines on glutamate reuptake and release, inflammatory cytokines can also lead to the activation of the kynurenine pathway.[18, 19] The kynurenine pathway involves stimulation of the enzyme indoleamine dioxygenase which breaks down tryptophan into kynurenine. [18, 19] Kynurenine is then transported into the brain through the large amino acid transporter where it is converted to quinolinic acid (QUIN) in microglia as well as activated macrophages which can enter the brain during an inflammatory state. [12, 18-20] QUIN not only directly binds the glutamate [N-Methyl-D-aspartate (NMDA)] receptor, but also inhibits glutamate reuptake. [19, 21] Interestingly, in a recent study in rats, the NMDA antagonist ketamine was found to block the development of depressive-like behavior following administration of the inflammatory stimulus lipopolysaccharide.[22] Surprisingly, ketamine (a drug that has also shown profound efficacy in patients with treatment resistant depression)[23] had no effect on the inflammatory response or activation of the kynurenine pathway in LPS-treated animals, indicating that the “end game” of inflammation and activation of the kynurenine pathway may indeed be glutamate.[22]

Consistent with impact of inflammation on glutamate metabolism in laboratory animals, recent work by our group has shown that administration of the inflammatory cytokine, interferon (IFN)-alpha, to patients with hepatitis C leads to increases in CNS glutamate in the basal ganglia and dorsal anterior cingulate cortex (dACC) as measured by magnetic resonance spectroscopy (MRS).[24] The basal ganglia and dACC are well known targets of inflammatory cytokines on the brain, and cytokine effects on these brain regions have been associated with symptoms of fatigue and cognitive dysfunction as well as reduced motivation.[25] Increases in glutamate as measured by MRS correlated with both symptoms of fatigue (including reduced motivation) and peripheral markers of inflammation including sTNFR2.[26] Interestingly, our most recent data indicate that in patients with major depressive disorder, there is a stepwise relationship between CRP and basal ganglia glutamate with patients with a CRP >3mg/L, which is considered high inflammation, exhibiting significantly higher basal ganglia glutamate than patients with a CRP<1mg/L.[27] In addition, similar to what was found in IFN-alpha-treated patients, increased basal ganglia glutamate in depressed patients was correlated with decreased motivation and cognitive dysfunction as manifested by psychomotor slowing.[27] Relevant to the translational relevance of the potential relationships among inflammation, glutamate and fatigue and cognitive symptoms, a recent study in cancer patients receiving whole brain radiation demonstrated that the glutamate antagonist memantine was able to attenuate the effects of radiation on cognitive function, indicating that blocking glutamate may treat symptoms in cancer patients with an increased inflammatory load.[28] Like memantine, riluzole is a glutamate stabilizing agent that increases the reuptake of glutamate and is used for treatment of amyotrophic lateral sclerosis.[29] Riluzole has also been shown to improve symptoms of depression while decreasing extracellular glutamate in preclinical models and human subjects.[29] Of note, riluzole-induced decreases in glutamate in bipolar depressed subjects were observed within 1 week of riluzole administration.[29] Taken together, the data support that:

- A significant percentage of breast cancer patients have symptoms of fatigue and cognitive dysfunction during and after treatment.
- Inflammation has been associated with symptoms in breast cancer patients during and after therapy.
- Inflammation activates a cascade of events that can lead to excess CNS glutamate which can be measured by MRS in humans.

- Excess glutamate has been associated with multiple symptoms related to fatigue and cognitive dysfunction including decreased motivation and psychomotor slowing.
- Riluzole is a drug that can rapidly (within 1 week) reduce extracellular glutamate (quantified by MRS) and like memantine, a glutamate receptor antagonist, may reduce symptoms of fatigue and cognitive dysfunction in breast cancer patients.

No study has examined whether riluzole can reverse inflammation-induced glutamate changes and the associated behavioral alterations in breast cancer patients. Based on our preliminary data and previous literature, there is reason to believe that riluzole may lower glutamate in breast cancer patients with increased CNS glutamate, and by lowering glutamate reduce fatigue and other related symptoms including decreased motivation and psychomotor slowing. Therefore, the goals of the proposed research are to test the following hypotheses:

- Compared to placebo, the glutamate stabilizing drug riluzole will decrease CNS glutamate in a population of breast cancer survivors with fatigue and high inflammation (Aim 1).
- Decreased CNS glutamate in breast cancer patients treated with riluzole will be associated with decreased symptoms of fatigue and cognitive dysfunction including anhedonia and psychomotor slowing (Aim 2).

4. Objectives:
The primary objective of the proposed research is to provide the first data on the role of CNS glutamate and symptoms of fatigue in breast cancer patients using MRS and a medication that has been shown to lower CNS glutamate in animal models and human subjects. No previous study has examined the potential connection between increased inflammation, increased CNS glutamate and symptoms in breast cancer patients, although there is strong clinical and preclinical support for an important interrelationship among these variables. Identification of a significant relationship between increased CNS glutamate and symptoms will:

- Enable the development of inflammatory biomarkers to identify patients with altered CNS glutamate.
- Help focus future studies using glutamate stabilizing medications and glutamate antagonists on patients most likely to respond to glutamate-targeted therapies (personalization of trials and treatment).
- Expand treatment studies to include synergistic or sequential targeting of inflammation and glutamate to reduce symptom burden in breast cancer patients.

This study will also serve as a foundation for efforts to link the impact of inflammatory cytokines and their relationship with increased CNS glutamate and behavior to a variety of cancers. Moreover, by testing novel treatment approaches (targeting glutamate), may ultimately improve the quality of life of breast cancer and other cancer patients.

5. Specific Aims:
Breast cancer patients experience significant behavioral alterations including fatigue and cognitive dysfunction that persist in up to 30% of patients for months to years after therapy. Data from our group and others have demonstrated that alterations in behavior both during and after breast cancer treatment are associated with increased inflammation. One mechanism that may mediate the relationship between inflammation and behavioral changes in breast cancer patients is central nervous system (CNS) glutamate. Recent work from our laboratory using

magnetic resonance spectroscopy (MRS) has found that chronic exposure to the inflammatory cytokine interferon (IFN)-alpha leads to increased CNS glutamate [as reflected by the glutamate/creatine (Glu/Cr) ratio] which correlated with symptoms of fatigue and cognitive dysfunction. Similar relationships among increased inflammation (as measured by CRP), increased CNS glutamate and symptoms of anhedonia and psychomotor slowing were also observed in patients with depression. Glutamate changes are likely secondary to the capacity of inflammatory cytokines to block glutamate reuptake and increase glutamate release from astrocytes, while also activating the kynurenine pathway, leading to increased quinolinic acid, which can bind glutamate receptors and block glutamate reuptake. Excessive glutamate can in turn lead to neuronal damage.

Thus, CNS glutamate may serve as a final common pathway to CNS pathology and behavioral and cognitive disturbances in breast cancer patients after treatment. Interestingly, memantine, a drug that antagonizes glutamate signaling was found to preserve cognition in cancer patients undergoing whole brain radiotherapy, indicating that blocking glutamate may have neuroprotective effects under conditions of an increased inflammatory load. Taken together, these data suggest that glutamate may be an outstanding target for reversing the behavioral pathology associated with inflammation during breast cancer and its treatment. Demonstration that targeting glutamate to reverse fatigue and cognitive changes in patients with high inflammation and could potentially help treat the long-term toxicities of breast cancer and its treatment. The goal of the current proposal is to test the hypothesis that reduction of glutamate in breast cancer survivors with fatigue and high inflammation will be associated with decreased CNS glutamate and decreased symptoms of fatigue and cognitive function including anhedonia and psychomotor slowing. To test these hypotheses the following aims are proposed:

Aim 1: To study the impact of 8 weeks of riluzole treatment on CNS glutamate concentrations in breast cancer patients with increased fatigue and high inflammation. Using a double-blind randomized controlled trial design, 40 female breast cancer patients with increased fatigue (≥4 on a 10 point scale) and high inflammation (CRP>3mg/L) will be treated for 8 weeks with either riluzole or placebo (n=20 per group). At baseline and at 1 and 8 weeks of treatment, all subjects will undergo single voxel MRS to measure CNS glutamate. Voxels will be placed in the right and left basal ganglia and the dACC. In addition, chemical shift MRS imaging will be employed to explore potential glutamate changes in other brain regions of interest.

Hypothesis 1: Breast cancer patients administered riluzole will exhibit greater decreases in basal ganglia and dACC glutamate compared to women treated with placebo.

Aim 2: To determine whether decreased CNS glutamate correlates with improved symptoms of fatigue and cognition dysfunction. Behavioral assessments of fatigue and cognitive function including measures of motivation and psychomotor processing speed, attention, executive function, and memory will be assessed in the patients indicated in Aim 1 and correlated with glutamate measures in the basal ganglia and dACC as well as other brain regions as an exploratory analysis using chemical shift imaging.
Hypothesis 2: Decreased glutamate in the basal ganglia will be associated with decreased fatigue and improved motivation and processing speed. Decreased glutamate in the dACC will be associated with improved executive function and improved memory performance.

The proposed research will help elucidate a novel pathway to behavioral pathology in patients with breast cancer, while also identifying peripheral biomarkers of risk as well as a novel treatment.

6. Study design and methods:

Participant Selection
Breast cancer research participants will be referred by physicians in the Glenn Family Breast Center at Winship Cancer Institute of Emory University, recruited through social media campaigns, or recruited through letters from the Georgia Center for Cancer Statistics. We plan to enroll 120 female breast cancer patients between the ages of 21-65 inclusive (to obtain 40 patients with analyzable data). All breast cancer patients will have completed surgery (lumpectomy or mastectomy) with or without neoadjuvant or adjuvant chemotherapy and with or without radiation between 1-5 years prior to enrollment.

Participants
40 female patients with Stage I-III breast cancer that are between 1-5 years post treatment and are between the ages of 21 and 65, unless otherwise approved by the PI, will be enrolled in this clinical trial. Participants will have completed surgery (lumpectomy or mastectomy) with or without neoadjuvant or adjuvant chemotherapy and with or without radiation. Subjects must have a CRP>3mg/L and a Single Item Screening Scale for Fatigue score of ≥4 (out of 10 points, 0 being no fatigue and 10 being severe, incapacitating fatigue) to be eligible for the trial. Based on projections that ~50% of recruited subjects will have a CRP>3mg/L and a Fatigue score ≥4 and ~20 of subjects will drop out during the clinical trial, we anticipate that we will need to recruit 120 subjects to yield analyzable data for 40 breast cancer subjects. No patients will be enrolled from vulnerable populations, including neonates, children, prisoners, or institutionalized individuals. No use will be made of fetal tissue. Potential subjects will be excluded for a number of medical conditions that might represent a risk for riluzole (including history of allergic reaction to riluzole and evidence of liver disease) and potentially confound the relationship among CNS glutamate, inflammation and behavior/cognition, including autoimmune or inflammatory disorders, chronic infectious diseases (e.g. HIV, hepatitis B or C), pregnancy, neurologic disorders (including a history of serious head trauma or seizures), liver disease (as manifested as an elevation in liver transaminases) and uncontrolled cardiovascular, metabolic, pulmonary or renal disease (as determined by medical history and laboratory testing). Subjects with a current or past history of schizophrenia will also be excluded. Subjects with Bipolar Disorder that have experienced a manic episode within 6 months of study entry will be excluded or at the PI's discretion. Subjects receiving antidepressants, mood stabilizers, antipsychotic medications or

benzodiazepines or drugs known to affect the immune system (e.g. glucocorticoids, methotrexate) will also be excluded,unless otherwise approved by the PI. Subjects who exhibit signs of an infection at screening will be rescheduled when symptoms have resolved. Subjects who exhibit signs of infection or a significant change from baseline test measurements via safety labs will be informed. Her treating physician may be notified for follow-up or results will be used by study clinicians to determine appropriate medical care in the case of adverse events and/or study continuation.

Compensation: For the main riluzole study, participants will be compensated at the end of each study visit using ClinCard. The ClinCard is a web based, reloadable, debit card that automates reimbursements for clinical research participants. However, if ClinCard accessibility is not available, participants may be compensated in the form of cash, check or gift card. Participants will receive $25 for each screening visit (which could be up to 2 visits) or $50 if they complete the consent and all of the screening assessments in a single visit, $25 for each completed study visit and $250 for each visit including an MRS scan, if scan is completed, for compensation for their time and effort. Participants will receive compensation for completed MRS scans upon completion of study participation. Participants will also receive $25 for completing cognitive tasks for Baseline I and Baseline II, and $50 for completing cognitive tasks for Week 8. Participants will receive additional compensation for completing the Effort-Expenditure for Rewards Task and earnings will be rounded to the nearest $5. If participants do not finish the study, we will compensate them for the visits completed. Participants can receive a maximum of $1100 for study completion. If there is a considerable break between study visit procedures (i.e. a lapse of at least 2 hours outside of the structured study schedule), the participant may leave and then return to complete the visit and will be compensated an additional study visit's compensation of $25. Further compensation (up to an additional $25 per visit) will be provided to cover travel expenses if participants live equal to or greater than 50 miles outside of the Atlanta city limits. Payments will be provided as follows: $25 in cash for the screening visit (s), $25 in cash for each study visit, $25 in cash for neurocognitive testing at Baseline I and Baseline II, and $50 in cash for neurocognitive testing at Week 8. The additional compensation for the Effort-Expenditure for Rewards Task will be given in cash for each study visit when the task is completed. The remaining $750 will be mailed to participants in a check at the end of study completion. Each participant will be asked to fill out a tax form, including their Social Security or Taxpayer Identification Number, in order to be reimbursed, depending on the amount and method of payment. Some payment methods involve mail coming to a participant's home address, which may be seen by others members of the household. Participants can decline payment if concerned about confidentiality or will be told to discuss other payment options with the study team. For the startle study, participants will be compensated an additional $50 per study visit for your time and effort, totaling to $100 if all parts of the sub-study are completed in two visits.

Recruitment and Informed Consent

Female subjects with breast cancer will be recruited from local (inter-Departmental) referrals, medical record queries, and/or completion of a RedCap survey in response to an IRB approved social media recruitment campaign(s). Letters will also be sent from the Georgia Center for Cancer Statistics to breast cancer survivors who are potentially eligible for the study. If those women are interested, they may sign the consent for contact information release through the

Georgia Center for Cancer Statistics, after which trained study personnel may contact them for study recruitment. An overview of the study will initially be provided in person or over the phone by study clinicians or trained study staff. A telephone prescreening interview will be conducted on candidates providing verbal consent. If a subject shows interest in the study, research staff members will describe the general procedures involved and will answer relevant questions. If a subject remains interested in participation, the detailed nature, purpose, procedures, benefits, risks of, and alternatives to this research study will be explained to each subject and written informed consent will be obtained by the study clinician who provides this information. Informed consent will be documented on the Institutional Review Board-approved form. A copy of the signed consent form will be given to the participant and the original document filed in a central study consent binder. The consent binder(s) and subject casebooks containing information gathered as part of the study will be kept in a locked office and/or cabinet.

Verbal Consent to Verify Breast Cancer Information

For participants who did not undergo their breast cancer treatment within Emory Healthcare, they may verbally consent to having their diagnosis and treatment information (e.g. cancer stage, nodal involvement, types and dates of treatment, and receptor status) verified through the Georgia Center for Cancer Statistics. This verbal consent process will be recorded on an IRB-approved verbal consent form by trained study personnel. The study clinician may also obtain a written informed consent.

Telephone Prescreen Interview

A telephone prescreen interview will be conducted with study referrals following verbal consent. Candidates eligible to proceed with the study process will be scheduled for an appointment to see a study clinician.

Onsite visit (s): Trained personnel will obtain written informed consent from candidates prior to initiating study procedures. Screening information will be collected by interview and evaluation, as well as obtaining collateral history and data from other relevant sources (e. g. medical records, referring physician).

Screening will include the following assessments: (1) History of medical and psychiatric conditions (2) screening laboratory evaluations, (3) subject height and weight measurements and waist circumference obtained by staff 4) Concomitant Medications 5) Adverse Events 6) MRI Safety Form 7) Demographics and 8) assessments below:

Mini-Mental State Exam (MMSE) is a 27-item interviewer administered questionnaire widely used for the evaluation of general cognitive functioning and identification of altered mental status. At screening, subjects will be excluded for score ≤28 (unless otherwise approved by the PI), which is evidence of mild cognitive impairment.[30]
WRAT-3 is a very brief screening measure for reading level. Participants evaluated at less than an 8th grade reading level may not participate unless otherwise approved by the PI.
Child Trauma Questionnaire (CTQ) is a standardized, retrospective 28-item self-report inventory that measures the severity of different types of childhood trauma. Participants will not be excluded for history of childhood trauma.

Description of Study Procedures (Baseline 1, Startle Study, Baseline 2 and Weeks 1-8)

All eligible participants with a CRP>3mg/L and a Fatigue Score ≥4 will complete Baseline 1, be randomized to receive riluzole (100 mg/d) or placebo atBaseline 2, and proceed to study visits at Weeks 1, 2, 4, and 8.
During the Baseline 1 visit and on Week 1 and Week 8, subjects will undergo magnetic resonance spectroscopy (MRS). Between baseline 1 visit and baseline 2 visit, participants will have the option of undergoing a parallel Startle Test Study protocol. This protocol will consist of either 1 or 2 visits, for completing psychiatric assessments and self-report forms and for completing a psychophysiological startle task. A schedule of all assessments is provided in Table 1. Approximately 30 mL of blood will be drawn at the same time of day between 8-10AM (to reduce potential circadian effects) under sterile conditions at Baseline 1 and 2 and Weeks 1, 4 and 8. Screening/safety labs are not time dependent. Safety labs will be completed at Baseline 2, Week 4 and Week 8. At each visit, self-report forms with urine toxicology will occur as well as recording of concomitant medications and adverse events. Neurocognitive testing will be completed on Baseline 1, Baseline 2 and Week 8. Where indicated, participants will be administered the neurocognitive tests and the scanning sequence of the MRS will be completed based on scanner availability and at the discretion of the PI or PI's designee. Subject will be read MRI instructions 48hrs prior to Baseline 1, Week 1, and Week 8 visits to have the subject avoid specific foods, medications, alcohol and cigarettes starting 24hrs prior to the MRS scan. A light lunch/snack may be provided after the fasting lab work.

Table 1. Schedule of Study Procedures and Assessments

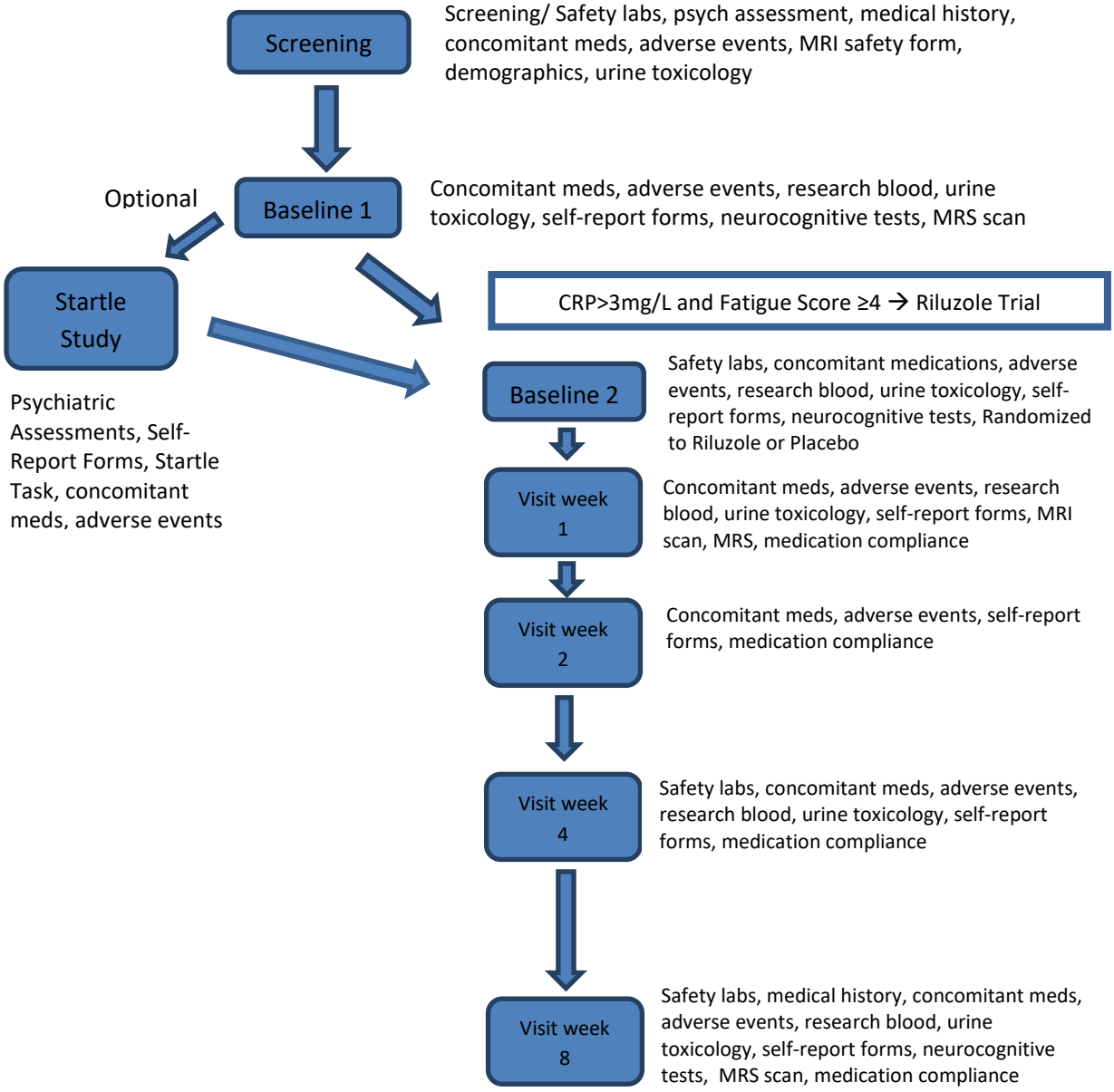
Procedures for MRS Controls	Intake	Screen Visit	Baseline 1[+]	Startle Study (optional)	Baseline 2[+] (randomization)	Week 1[+]	Week 2[+]	Week 4[+]	Week 8[+]
Informed consent	X								
Screening/ Safety labs*		X			X			X	X
Psychiatric Assessments*, **		X		X					
Medical History and Physical		X							X
Concomitant Medications		X	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X	X	X
MRI Safety Form		X							
Demographics		X							
Startle Task**				X					
Research Blood*			X		X	X		X	X
Urine toxicology (Test kit)		X	X		X	X		X	X
Self-Report Forms*, **			X	X	X	X	X	X	X
Neurocognitive Tests*			X		X				X
MRI Scan Food, Drink and Cigarette Intake Form			X			X			X
MRS			X			X			X
Placebo or Riluzole (100mg/d)					X	X	X	X	X

[+] - for patients with a CRP>3mg/L and a Single Item Screening Scale for Fatigue Score ≥4
*** - for patients completing the main branch of the Riluzole Study**
**** - for patients who opt for to complete the Startle Test Study**

Key:
Main Riluzole Study
*Screening/ Safety Labs: CBC w/Diff, Metabolic Panel, hCG quantitative serum, TSH, Urinalysis w/micro at Screening and Week 8 only, Urine drug test all visits (test kit), except week 2 visit; Testing for CRP, Hepatitis B and C and HIV will be conducted on the screening visit only, unless otherwise approved by the PI. A Rapid C Reactive Protein finger prick may also be obtained by trained research staff using a rapid CRP finger prick test at the discretion of the investigator to expedite determining eligibility for research participation.
*Psychiatric Assessments: WRAT-3, MMSE, CTQ, QID-S, Single Item Screening Scale for Fatigue
*Research Blood: Cytokine Multiplex, TRP, KYN, mRNA analysis, methylation analysis, and gene analysis (collected under fasting conditions)
*Self-Report Forms: IDS-SR, MFI, PROMIS-Fatigue, BAI, PSQI, Assessment of Pain level, PHQ-9, Single Item Screening Scale for Fatigue, Baycrest Memory Scale
*Neurocognitive Tests:
Baseline 1 - EEfRT,Finger Tapping Task (FTT), The Trail Making Test (TMT), Digit Symbol Task, Reaction Time Task (CANTAB)
Baseline 2 - Rey Auditory Verbal Learning Test (RAVLT), Delayed Matching To Sample (CANTAB), Spatial Working Memory (CANTAB), Stroop Color and Word Test
Week 8- EEfRT, Finger Tapping Task (FTT), The Trail Making Test (TMT), Digit Symbol Task, Reaction Time Task (CANTAB), Rey Auditory Verbal Learning Test (RAVLT), Delayed Matching To Sample (CANTAB), Spatial Working Memory (CANTAB), Stroop Color and Word Test

Startle Test Study
**Psychiatric Assessments: CAPS, MINI, MPSS
**Self-Report Forms: Demographics Form, Menstrual Cycle Questionnaire, CD-RISC, BDI-II, TEI, Life Events Checklist, Post Traumatic Growth Inventory, Saliva Pre-collection Questions, Post-testing questions
**Startle Task: Electromyographic activity, Acoustic startle, Electrocardiogram activity, Electrodermal activity, Response keypad, Sniffin' Sticks, Saliva Collection

Riluzole Flowchart:



Randomization

Subjects will be randomized to 100 mg of riluzole (50 mg po BID) or an identical appearing placebo in a 1:1 fashion with no stratification. Subjects will be instructed to take the study medication on an empty stomach (1 hour before or 2 hours after meals). The dosage of riluzole is based on several clinical trials that have previously used this medication to treat a number of psychiatric disorders ranging from depression to generalized anxiety disorder as well as ALS.[31] A randomly generated sequence of assignments to riluzole or placebo will be created and maintained by the study pharmacist. Subjects will be randomized in blocks of 4. If a subject drops out prior to receiving the first dose of medication, she will not be included in data analyses and her randomization assignment will "roll over" to the next recruited subject until 40 subjects have received at least one dose of medication or placebo.

Compliance

Patients will be asked to fill out a medication log and bring in their pill bottles at each study visit. Patients who have taken less than 75% of their medication may be excluded from study participation. If study removal occurs at or after the Week 4 study visit, the patient will undergo exit safety laboratory testing and physical examination.

Main Riluzole Study

Psychiatric Assessments

Quick Inventory of Depressive Symptoms (QIDS) (healthy controls only) is a 16 item self-report instrument that includes assessment of nine diagnostic symptom domains used to characterize a major depressive episode. At screening, subjects will be referred to PI or PI's designee for a QIDS score ≥16.
Childhood Trauma Questionnaire (CTQ) is a clinician-assisted inventory used to assess 3 types of childhood abuse: sexual, physical, and emotional.
Mini-Mental State Exam (MMSE) is a 27-item interviewer administered questionnaire widely used for the evaluation of general cognitive functioning and identification of altered mental status. At screening, subjects will be excluded for score ≤28 (unless otherwise approved by the PI), which is evidence of mild cognitive impairment.[30]
WRAT-3 is a very brief screening measure for reading level. Participants evaluated at less than an 8th grade reading level may not participate unless otherwise approved by the PI.

Self-report Forms

Single Item Screening Scale for Fatigue is a single question self-report screening instrument that asks subjects to rate their fatigue on a scale of 0-10 where 0 = No fatigue and 10 = Worst fatigue you can imagine. Ratings are categorized as none to mild (0–3), moderate (4–6) or Severe (7–10). This screening strategy for fatigue is recommended by the National Comprehensive Cancer Network (NCCN) Guidelines.
Pain assessment scale is a Likert style self-report pain measurement (10 items; 1=no pain; 10=extreme pain) to be completed by breast cancer participants to gauge their ability to lie comfortably in the scanner. Participants reporting a current pain level of $\geq$5 may not be approved to participate unless otherwise approved by the P.I.
Inventory of Depressive Symptomatology-Self Reported (IDS-SR) is a 30-item self-report instrument with excellent psychometric properties that was designed to measure symptom constructs consistent with current psychiatric diagnostic nosology and has been widely used as a self-report outcome measure of depression in treatment trials.[32, 33] Of note, the IDS-SR is

the patient reported version of the IDS-C used in our other breast cancer study. In the event that a subject becomes unduly distressed or scores >32 on the IDS-SR (indicating moderate to severe depressive symptoms) or endorses a score >2 on the suicide item of the IDS-SR (Question 18), the PI or his designee will be immediately contacted.

Multidimensional Fatigue Inventory (MFI) is a 20-item scale used to evaluate the presence and severity of fatigue among subjects by self-reports.[34] The MFI assesses 5 dimensions of fatigue, including general fatigue, physical fatigue, mental fatigue, reduced activity, and reduced motivation. The MFI has been used by our group to quantify fatigue in breast cancer patients, and a minimal clinically important difference of 10 points has been established.[35, 36]

PROMIS-Fatigue Short Form is a 7-item scale developed by the Patient-Reported Outcome Measurement Information System (PROMIS), a part of the NIH Roadmap Initiative which is focused on developing a publicly available resource of standardized, accurate, and efficient outcome measures of symptoms, distress, and functioning. The criterion for a minimally clinically important difference in patients with advanced-stage cancer is a 3 to 5 point difference in raw score.[37] Recommendations for high priority research on cancer-related fatigue recommend use of the PROMIS fatigue scale to allow comparison of results across studies.[38]

Pittsburgh Sleep Quality Index (PSQI) will be used to assess sleep quality over the past month. The scale contains 15 multiple-choice items that inquire about frequency of sleep disturbances and subjective sleep quality and 4 write-in items that inquire about typical bedtime, wake-up time, sleep latency, and sleep duration. The PSQI generates seven scores that correspond to relevant domains of sleep, and each score ranges from 0 (no difficulty) to 3 (severe difficulty). The component scores are summed to produce a global score (range of 0–21).

Beck Anxiety Inventory (BAI) is a 21-item self-report measure of anxiety-related symptoms, rated on a 4-point Likert scale based on the patient's experience in the last two weeks.

Patient Health Questionnaire (PHQ-9*)* is a nine-item measurement used to assess depressive symptoms and suicidal ideation. Study psychiatrists will be alerted to any participants scoring $\geq$ 3 on the suicidal item.

Baycrest Memory Scale is a 26 item memory scale developed by the Baycrest Centre for Geriatric care.

Neurocognitive Tests

The following neurocognitive test battery was developed to test domains of functioning and anatomical regions of interest relevant to the proposed Aims and Hypothesis.

Baseline 1:

Effort-Expenditure for Rewards Task (EEfRT): In this effort-discounting variant, subjects are presented with a choice between two task-difficulty levels in order to obtain monetary rewards.[39] In each trial, subjects must make repeated manual button presses within a certain period of time. To succeed in each trial, subjects must meet or exceed the required number of button presses within the time allotted. Easy-task trials require 30 button presses within 7 seconds, while hard-task trials require 99 button presses within or before 21 seconds have passed. There are also probability cues attached to each trial—88%, 50%, and 12%—that determine whether a trial may result in compensation, upon completion. This information will be made known to the subjects at the start of the task. Upon completion of all trials, three of the trials will be randomly selected and the subject will receive compensation from those trials based on the choices they made. This payment may range from approximately $3.00- $24. This

task lasts approximately 20 minutes, and the number of trials subjects complete is based on their choices and button press rate.
The Trail Making Test (TMT) is a commonly used neuropsychological measure with updated norms that has two parts. Part A (TMT-A) requires the patient to connect numbered circles in order and reflects a measure of basic attention and processing speed, while Part B (TMT-B) requires the patient to alternate between numbers and letters and is considered a measure of executive function, including planning and organization.
Digit Symbol Task: The Digit Symbol Task is a subtest of the Wechsler Adult Intelligence Scale (WAIS) and consists of rows of blank squares, each printed with a randomly assigned number (1-9). A key is printed above these rows and shows each number paired with a different nonsense symbol. The subject's task is to fill in the blanks with the corresponding symbols as rapidly as possible. This test involves graphimotor speed, visual scanning and memory, with about half of the variance being accounted for by graphimotor speed, a third by visual scanning and 4-5% by memory [40]. As part of the WAIS, this test is frequently used in neuro-psychology and relevant norms and test-retest reliability have been well established [41]. Performance on the Digit Symbol Test has been found to correlate with subcortical atrophy (esp. as measured by the bi-caudate ratio) in disorders involving the basal ganglia including Huntington's disease and multiple sclerosis.[42, 43]
Finger Tapping Task (FTT): This task uses a specially adapted tapper that the subject is asked to tap as fast as possible using the index finger. The subject is given 5 consecutive 10-second trials for both the preferred and non-preferred hands. The finger tapping score is the mean of the 5 trials and is computed for each hand.
Reaction Time Task (CANTAB): The reaction time test includes simple and choice reaction time tasks and is divided into 5 stages requiring increasingly complex chains of responses and providing distinction between reaction (or decision) time and movement latencies

Baseline 2:

Rey Auditory Verbal Learning Test (RAVLT): A test of verbal memory and learning using list learning that measures ability to use strategies to improve learning.
Delayed Matching To Sample (CANTAB):[44, 45] **Variables:** Response time, Discrimination (Hits/False Alarms). A measure of visual memory where subjects are asked to identify objects shown previously at various time points.
Spatial Working Memory (CANTAB):[46] **Variables:** Response time, Correct responses. A test of ability to remember spatial localization of objects presented previously
Stroop Color and Word Test: Subject is requested to name the color of a word both when the color of the ink matches the name of the color and when the color of the ink does not match the name of the color. Prolongation in reaction time and number of inaccurate responses during the name-ink color mismatch condition are measured.

Week 8:

Subjects will complete all neurocognitive testing from baseline 1 and baseline 2. Testing will be divided into two sessions with a break of at least ½ -1 hour.

Startle Test Study (Optional):
Psychiatric Assessments

Clinician Administered PTSD Scale (CAPS) is an interview-administered diagnostic instrument measuring PTSD. The CAPS assesses lifetime and current PTSD. It also yields continuous measures for the severity of both overall PTSD and of the three symptom clusters (intrusion, avoidance, and arousal). The frequency and intensity of scores for each of the 17 diagnostic criteria are summed to arrive at a total severity score.[47, 48]
Mini International Neuropsychiatric Interview (MINI) is a commonly used psychiatric structured diagnostic interview that will be used to assess psychiatric disorders. A cover sheet will be used to provide interviewers with an overview of participants' medical history.
Modified PTSD Symptom Scale (MPSS) is a 17-item interview used to aid in the detection and diagnosis of PTSD. The MPSS will be used in this study as a rapid assessment tool because of its reasonably good diagnostic validity for PTSD.[49, 50]

Self-Report Forms

Demographics Form (DF) covers demographics; family composition; living situation; and personal and family psychiatric medical, and substance abuse history; and income and education.
Connor-Davidson Resiliency Scale (CD-RISC) is a 25-item scale measuring ability to cope with stress and adversity. The CD-RISC demonstrates adequate internal consistency, test-retest reliability, and convergent and divergent validity.[51]
Menstrual Cycle Questionnaire is a self-report measure that covers menstrual cycle history.
Beck Depression Inventory II (BDI-II) is a 21-item measure of depression symptoms. It is a commonly used screening instrument for depressive symptoms.
Traumatic Events Interview (TEI) is an instrument assessing lifetime history of traumatic experiences including experiencing, witnessing, and being confronted with these stressors.
Life Events Checklist is a self report measure to screen for traumatic events in the participant's life.
Post Testing Questions will be used to assess participants.
Post Traumatic Growth Inventory is a 21-item scale that will be used to assess positive outcomes as a result of cancer survivorship.[52]
Post Testing Questions will be used to assess participants' perception of the tasks.
Saliva Pre-collection Questions will be used to assess quality of saliva collection.

Psychophysiological Task

Fear-Potentiated Startle*:* The following methods will allow us to assess fear acquisition, within- and between-session extinction, and conditioned inhibition (learned safety), as well as subjects' awareness of reinforcement contingencies in the experiment. The aversive stimulus (US) in these studies will be a 250 ms airblast with an intensity of 140 p.s.i. directed to the larynx as described in similar human fear conditioning studies. [53-55] Airblasts will be emitted by a compressed air tank connected to polyethylene tubing and controlled by a solenoid switch. Conditioned stimuli will be presented as visual stimuli according to our previously published methods and will consist of colored geometric shapes presented on a computer monitor mounted to the wall of the sound attenuated test chamber. [56] The CSs that are reinforced with an airblast US (CS+'s) and those that are not reinforced (CS-'s) will be counterbalanced across subjects. Stimuli will be presented using SuperLab 3.0 for Windows (Cedrus, Inc.) and

synchronized with psychophysiological data acquisition using a DIO card (Measurements Computing, Inc). This paradigm has been successfully performed previously in our group. [56]

Physiological Variables

Electromyographic activity: We will collect electromyography (EMG) recordings of the corrugator supercilii muscle (eyebrow muscle). According to the guidelines from Cacciopo and colleagues [57], two 5 mm Ag/AgCl electrodes will be placed above the right eyebrow. EMG activity will be acquired at a sampling rate of 1kHz, amplified and digitized using the EMG module of the Biopac MP150 for Windows (Biopac Systems, Inc., Aero Camino, CA).

Acoustic startle: The acoustic startle response (eyeblink component) will be measured via EMG of the right *orbicularis oculi* muscle. Two 5 mm Ag/AgCl pre-gelled disposable electrodes will be positioned approximately 1 cm under the pupil and 1 cm below the lateral canthus. The startle probe (noise burst) will be a 108-dB (A) SPL, 40-ms burst of broadband noise with a near instantaneous rise time.

Electrocardiogram activity: Heart-rate and heart-rate variability (HRV) will be measured using the ECG module of the Biopac system at a sampling rate of 1 kHz. One 5mm Ag/AgCl electrode will be placed on the chest above the right clavicle, another electrode will be placed on the chest under the left side of the ribcage.

Electrodermal activity: Skin conductance level and skin resistance data will be acquired at a sampling rate of 1 kHz using the GSR module of the Biopac system. Two 5 mm Ag/AgCl disposable electrodes filled with isotonic paste will be attached to middle phalanges of the second and fourth finger of the non-dominant hand.

Response keypad: To assess subject awareness and US expectancy during each experimental session subjects will respond on a response keypad (SuperLab, Cedrus Corp.) in coordination with the EMG startle response monitoring system (SR-LAB, San Diego Instruments). During the fear conditioning sessions subjects will press a button marked "+" if they expect a CS to be followed by the US, a button marked "-" if they do not expect a CS to be followed by the US, and a button marked "0" if they are uncertain of what to expect.

Sniffin' Sticks: Participants' ability to discriminate odor will be assessed based on identification of every-day odors using commercially available, "Sniffin' Sticks".[58]

Saliva Collection: Saliva samples will be obtained during the experiment. These will be stored in a -80°C freezer for hormonal analysis and DNA methylation analysis.

Reliability of Behavioral and Neurocognitive Ratings

The collection of WRAT-3, MMSE, CTQ and medical and psychiatric information (acute status and history) will be collected by trained study personnel. Dr. Michael Treadway, Assistant Professor of Psychology at Emory University, will train staff on the administration of, Effort-Expenditure for Rewards Task (EEfRT).

Laboratory Variables

Blood collection: Research bloods will be collected by venipuncture into EDTA-containing vacutainer tube using standard sterile technique. Plasma and buffy coat for the evaluation of concentrations of cytokines and their receptors as well as CRP will be obtained by centrifugation of whole blood at 1000 x g for 10 minutes at 4°C. Plasma and buffy coat will be removed

separately, aliquoted into siliconized polypropylene tubes, and stored at -80°C until batch assay. Bloods will also be collected by venipuncture into Tempus mRNA tubes and directly placed in freezer storage at -20C. At screening, CRP will be processed in Emory Medical Laboratories and/or using the Diazyme hsCRP POC Test Kit. This kit produces rapid results using a finger prick method to collect 20 µl of blood and is intended for the in vitro quantitative determination of C-reactive protein. Blood and blood plasma will be evaluated in the lab of Dr. Brian Dias at the Yerkes National Primate Research Center at Emory University for the evaluation of exosomes, RNA, and hormones. Tempus tubes and buffy coats will be sent to the Emory Integrated Genomics Core for RNA extraction, gene expression, mRNA profiling, epigenetic evaluation and measurement of gene polymorphisms.

Plasma cytokines and soluble cytokine receptors: Customized high sensitivity Fluorokine MAP Multiplex Human Biomarker Panels (R&D Systems, Minneapolis, MN) will be used to measure plasma TNF-alpha, sTNFR1 and 2, IL-1ra, IL-6 and sIL-6R. All samples will be assayed in duplicate according to manufacturer's instructions. Quality control plasma of both low and high cytokine concentrations will be included with every assay. The mean inter- and intra-assay coefficients of variation for control samples are reliably 10% or less.

C-reactive protein (CRP): Plasma CRP will be assessed with a high sensitivity turbidimetric assay. Sensitivity is 0.18 mg/L, range of measure is 0.2 to 80 mg/L, and functional sensitivity (at 20% CV) is 0.2 mg/ L.

Tryptophan and Kynurenine: Free tryptophan and kynurenine plasma concentrations will be determined by high-performance liquid chromatography in the laboratory of Dr. Dietmar Fuchs or other laboratories with equivalent expertise at the PI's discretion.

Gene expression and epigenetics: As indicated above, blood will be obtained for analysis of inflammatory mediators and signaling pathways.

Saliva Collection: Saliva will be collected after the startle task by asking participants to spit into two tubes. This sample will be analyzed for hormones and DNA methylation changes present at time of study visit.

MRI Scans

MRS acquisition and processing: All images will be acquired using a Siemens 3 Prisma 3T scanner (Siemens Medical Solutions, Malvern, PA, USA) equipped with a maximum gradient strength of 80 mT/m and a rise time of 200 µs, which will provide nearly double the signal-to-noise ratio enabling high-resolution structural imaging data. The new digital RF system equipped with 64-channel RF head-and-neck coil and the two-channel RF excitation coils will provide better spectral resolution with improved signal-to-noise ratio while at the same time providing more uniform excitation pulses. For image guidance and MRS volume of interest prescription, anatomic images will be obtained using a T1-weighted MPRAGE sequence with the following parameters: TR/TI/TE =2300/900/3.02 msec; flip angle=8°; FOV read=256 mm; matrix=256; slice thickness=1 mm; GRAPPA factor of 0; total scan time=10 minutes.

Single Voxel MRS Acquisition: Single Voxel Spectroscopy (SVS) is used to quantitate neurometabolites of interest in target brain regions. The scanning protocol and post-processing methodology will be used in accordance with previously published reports,[24, 27] but modified for the Prisma 3T platform. SVS will be acquired using Chemical Shift Selective (CHESS)-based MRS sequence using settings of TR/TE = 3000/30 ms, sampling size=128 averages, bandwidth=1048Hz with sampling size = 1024, 128 averages. Two voxels with size of 17 x 30 x 17 mm3 located in the left and right basal ganglia will be used for SVS. Spatial localization will be implemented using PRESS technique.[59] Numerically optimized Shinnar-Le Roux (SLR)

radio frequency pulses will be used for PRESS (90°, 180°, 180°) and CHESS (90°, 90°, 90°).[60] Four unsuppressed water FIDs (free induction decay) will be also acquired for eddy current and phase correction[61] and to compute metabolite/water ratios if needed (LC Model). The FWHM of the unsuppressed water peak will be optimized to <14 Hz by shimming. The spectral data will not be apodized.

Chemical Shift Imaging (CSI): The application of a single voxel to a specific region limits evaluation of diffuse or global cortical and subcortical glutamate abnormalities that may occur in association with inflammation and have been proposed to occur in unipolar depression.[62] Simultaneous sampling from multiple voxels placed bilaterally on the basal ganglia gray matter, white matter and adjacent neocortical regions will provide a more expansive evaluation of relevant brain structures that have been implicated in the impact of inflammatory cytokines on behavior. CSI will be acquired using 2D Point Resolved Spectroscopy (PRESS)-based MRSI sequence using settings of TR=1590 ms, TE=30 ms, averages= 7, matrix=16×16, voxel size=11. 3×11.3×15 mm3, slice thickness=15mm. The raw files will be processed using LC Model package-Version 6.2-2b.[63]

Post Processing:

Metabolite Spectral Fitting: The LC Model is an operator-independent commercial software package that fits in vivo metabolite spectra using model resonances acquired from multiple compounds in standard phantom solutions. The water suppressed time-domain data will be analyzed between 1.0 ppm and 4.0 ppm without T1 and T2 correction. The basis set provided by the vendor of the LC Model[63] will be used and then scaled to account for receiver gain differences. While the entire 18 metabolite basis set would be entered during metabolite fitting, the analysis will only include metabolite variances (Cramer Rao Lower Bounds or CRLB) <25. Use of a high field strength (Prisma 3T) scanner and short TE (=30 msec) sequence has enabled us to calculate Glu/Cr with high reliability and very low metabolite variance (CRLB <10) during our previously published MRS studies.[24, 27]

Water scaling and relaxometry: The post processing pipeline has been developed to yield absolute quantitation, metabolite/water and metabolite/creatine values. LC model provides an automated water scaling settings which will be used (DOWS). All data (water signal and water suppressed data) will be phased followed by quantification of water relaxometry data. The relaxometry data will then be fitted to obtain water reference data and in the case of gray matter following segregation of the CSF and tissue water constituent. Finally, water signal will be scaled to that of the resulting value obtained in previous steps. The water scaling portion of the post processing will be done as soon as data is acquired.

Tissue correction and segmentation: T1-weighted images will be segmented into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) compartments using FreeSurfer (https://surfer.nmr.mgh.harvard.edu/fswiki) on the whole brain T1 images. A volume of interest (VOI) will be generated on the T1 images, matching the location and size of MRS voxel. Volumes of GM, WM and CSF segments in this volume-of-interest will then be generated using FreeSurfer. The absolute metabolite concentrations generated by the LCModel will then be corrected for CSF using the formula metabolite C = Co x 1/(1-fCSF), where C is corrected metabolite concentrations, Co is metabolite concentrations generated by LCModel output and fCSF is fraction of CSF volume.[64, 65] The final output of the absolute metabolite quantitation will be provided in units of molality. Absolute quantitation using CSI data is challenged by multiple methodological problems[66] and often requires customized automated programs to correct for atrophic tissue changes, B0 field inhomogeneity distortions, and water suppression in each subvoxel may be less than optimal. Consequently, CSI data will be assessed using metabolite values normalized to creatine. This will be accomplished after ensuring that raw uncorrected concentrations of creatine (Cr) and phosphocreatine (PCr) are not significantly

different among the different study groups in the prescribed region of interest. An exploratory analysis of the impact of inflammation on other metabolites of interest including choline (Cho), myoinositol (InS) and n-acetyl aspartate (NAA) will also be conducted.

Management of MRS Incidental Findings
The purpose of these scans is not to make a clinical determination of patients' brain health. However, if any incidental finding is noticed during the scan, the information will be provided to patients or their medical provider-of-record for further management. Incidental findings are those abnormalities seen in patients' brain images during the scanning process, which may or may not be of clinical importance. Follow-up of the scan findings will be the responsibility of the patients' Emory Healthcare provider-of-record.

7. Data Quality Monitoring Plan:
Periodic chart monitoring will be conducted by study personnel to validate integrity of the data. Chart reviews will take place after each block of five subjects and may include, but not be limited to, the presence of a signed informed consent document, documentation that the informed consent was obtained prior to performance of any study-related evaluations, documentation related to billing compliance, treatment calendars, enrollment reported to OCR, enrollment recorded in Oncore ®, treatment order and/or prescription form copies, and source documentation as defined in the Winship Clinical Trials Office's Standard Operating Procedure.

The project will be subject to random chart audits conducted by the Winship Clinical Trials Office in accordance with Standard Operating Procedures. A minimum of 5 subject charts may be randomly audited as requested by the PI, the Associate Director for Clinical Research, the Director of the CTO, the IRB, the ORC, or other Emory research compliance personnel, or the assistant director on an as needed basis.

1. Any of the above persons may request a random audit of clinical trials records for the subjects enrolled on any study managed by this office.
2. When records are requested they will be made available to the person listed above within 2 hours.
3. Chart review may include, but not be limited to, the presence of a signed informed consent document, documentation that the informed consent was obtained prior to performance of any study-related evaluations, documentation related to billing compliance, treatment calendars, enrollment reported to OCR, enrollment recorded in Oncore ®, treatment order and/or prescription form copies, and source documentation.
4. Audit will be performed by the persons listed above in "Procedure" or the internal monitors.
5. Audit results may be reviewed by the Winship CTO Director, the Assistant Director of Regulatory/Compliance, the Assistant Director for Clinical Research and the Office of Research Compliance. Significant findings will be discussed with the nurse, coordinator, principal investigator, and Director.
6. Deficiencies will be corrected within five working days and presented to the Assistant Director for review at that time. A corrective and preventive action plan will be developed as indicated by the audit results.

Quality Improvement
Charts with significant deviations from Winship CTO policy will indicate the need for further audit of records belonging to the involved nurse or coordinator. Limited findings of noncompliance will signal a need for increased education and/or quality monitoring. Significant noncompliance can lead to disciplinary action.

Failure to comply with this policy may be grounds for discipline by the University. Any disciplinary action taken by the University will follow the rules governing the individual's employment category.

8. Sources of Materials:
Research materials to be obtained will include clinician-administered psychiatric assessments, neurocognitive assessments, self-report questionnaires, blood sampling, MRS scans, physiological variables, and saliva sampling, Material for evaluating baseline medical status will be obtained from blood, urine, patient report and their medical record when available. Data to be recorded from subjects will include standardized ratings of fatigue, depression and cognitive function, as well as clinical information derived from medical records regarding tumor, patient, and treatment characteristics. Data to be obtained from peripheral blood will include complete metabolic panel; CBC with differential; hCG quantitative serum; plasma concentrations of cytokines and their soluble receptors, gene expression, gene polymorphisms, exosome, and/or hormonal anaylsis. Data obtained from urine will include urinalysis and toxicology screen. Data obtained from saliva will be used to assess hormone levels and DNA methylation changes at time of study visit. Clinical data will be used to assess eligibility and safety and will be collected via interview and documented by study clinicians. Standardized clinician-administered and self-report assessments will be administered and collected by study physicians or his designees. Blood will be obtained in the clinic laboratories by trained phlebotomists, and the samples will be collected and processed by study personnel.

All data, including questionnaires and blood will be coded by unique identifying numbers. Subjects' names will not be linked to their biological samples or clinical data in REDCap. Subject name and identifying number will be kept in a casebook in a locked cabinet and/or office. Only IRB qualified research staff and study physicians will have access to subject identifiers. Research personnel analyzing blood or clinical data will not be provided information from which they could identify subjects. All information will be obtained solely to determine eligibility for study participation or for research purposes. Nevertheless, any significant abnormalities uncovered will be reported to the subject and his/her treating physician for follow-up or will be used by study clinicians to determine appropriate medical care and/or study termination in the case of adverse event

9. Data Collection and Management:
The data management team will use REDCap.[67] REDCap allows database construction, an interface for collecting data, data validation, and automated export procedures for data downloads to statistical packages (SPSS, SAS, Stata, R). Scales will be completed securely via the internet via standard web access. Research staff will enter scale responses directly into the REDCap electronic assessment forms. Electronic assessments will be completed on computer terminals under supervision of study staff. Dr. Miller is currently using REDCap for 2 of his funded projects, and he and his staff have extensive experience with its usage. Data will be stored indefinitely in a secure location until no longer needed by investigator.

10. Statistical Analysis:

Aim 1: To study the impact of 8 weeks of riluzole treatment on CNS glutamate concentrations in breast cancer patients with increased fatigue and high inflammation.
Hypothesis 1: Breast cancer patients administered riluzole will exhibit greater decreases in basal ganglia and dACC glutamate compared to women treated with placebo.
Mixed-effects models for repeated measures (MMRM) will be employed to examine effects of group, time and their interaction on left and right basal ganglia glutamate or dACC controlling for baseline glutamate concentrations in separate analyses for each brain region. Relevant covariates including age, sex, race (white/non-white) and body mass index (BMI), socioeconomic status (as measured by household income), educational status, tumor stage, tumor receptor status (where appropriate), timing (adjuvant versus neoadjuvant) of chemotherapy and radiation (yes/no) will be sequentially tested in the models for goodness of fit (Akaike Information Criterion) and included as appropriate.

Aim 2: To determine whether decreased CNS glutamate correlates with improved symptoms of fatigue and cognition dysfunction.
Hypothesis 2: Decreased glutamate in the basal ganglia will be associated with decreased fatigue and improved motivation and processing speed. Decreased glutamate in the dACC will be associated with improved executive function and memory performance.
Random intercept linear mixed models will be used to assess whether change in left or right basal ganglia or dACC glutamate predict change in fatigue scores or change in neurocognitive function over time. Models will be adjusted for relevant clinical covariates (including IDS-SR, PSQI, BAI) as indicated above. Separate analyses will be run for each behavioral outcome (MFI, PROMIS) as well tests of cognition (HVLT-R, TMT PASAT COWAT RT FTT, RTT, and DSST) and motivation (DDT, EEfRT, Subjective Value Task, Effort and Delay Task, Daily Assessment/Mobile App Task, Gamble Task, and Go No-Go Variant Task.). Type I errors as a result of multiple testing will be controlled by using a step down Bonferroni procedure.[68]

11. Power Analysis:

A power analysis was conducted using our preliminary data examining a cohort of 50 unmedicated patients with major depression.[27] The study sample size was powered on the basis of Aim 1, which represents the primary hypothesis of the study that riluzole will decrease basal ganglia glutamate. Given a sample size of 40, an alpha of 0.05, a power of 0.80, we will be able to detect an effect size of Cohen's d=0.5 which falls in the moderate range (computed using G*power). This effect size is well within the range of the effect size (d) of the differences in basal ganglia glutamate concentrations between high (CRP>3mg/L) and low (CRP<1mg/L) inflammation, which are expected in this study.

12. Potential Study Risks:

Main Riluzole Study Risks: There are 4 major areas of potential risk in the proposed study stemming from 1) neuropsychiatric assessments, 2) blood drawing, 3) MRS scanning and 4) riluzole treatment. Neuropsychiatric assessments may uncover unpleasant feelings about the subject's present emotional state. The risks of blood drawing include discomfort, bruising, infection, bleeding and fainting. Undergoing MRS scan poses no more risk than undergoing a routine MRI scan. Nevertheless, physical discomfort due to lying in the scanner, occasional headaches due to scanner sounds and previously unrecognized claustrophobic attacks are the

prominent adverse effects of the procedure. Major complications of riluzole (Rilutek) include an allergic reaction to riluzole or the components of the tablet and hepatic toxicity. Riluzole is contraindicated in individuals who have a history of hypersensitivity reactions to riluzole or any of the tablet components and in individuals who have hepatic disease or who have baseline transaminases greater than 3 times the upper limit of normal. The drug is also contraindicated in women who are pregnant. Subjects of childbearing ability must agree, with the study doctor or PI's designee, on a method of birth control to use throughout the study. To ensure no contraindications with alcohol, participants will be told not to consume more than 1 alcoholic drink per day while on study medication (12 fl oz of beer, 5 fl oz of wine, 1.5 fl oz shot of distilled spirit, and also not to consume any alcohol within 24 hours of a study visit brain scan. Of the most commonly observed adverse events associated with the use of RILUTEK in 1,396 individuals with amyotrophic lateral sclerosis, those reported more frequently than with placebo treatment were: asthenia, nausea, elevations in liver function tests, dizziness, decreased lung function, diarrhea, abdominal pain, pneumonia, vomiting, vertigo, circumoral paresthesia, anorexia and somnolence. Table 2 shows the most common treatment-emergent adverse reactions considered possibly related to medication and for which the incidence was ≥1% in an open label Phase IV confirmatory trial for amyotrophic lateral sclerosis of 414 patients.

Startle Study Risks:
The most common risks and discomforts expected in this study are:

Risks associated with interviews: Psychological tests and interviews can sometimes bring up painful emotions. These emotions may include sadness, worry, or increased anxiety. If you have trauma-related stress symptoms, you may have an increase in nightmares or flashbacks related to your traumatic experiences. You are free not to answer any questions you wish. You may stop participating in the study at any time. This will not in any way affect your future care.

Risks associated with the acoustic startle test: During the startle measurement, scrubbing your skin with cleanser or application of skin tape may cause skin irritation. The noise level you will hear during the startle test session is about what you hear on a train. For most people, this sound level is not uncomfortable. If the sounds or airblasts cause you discomfort, you can stop the test session at any time. Withdrawal from the study will not affect your future care. There also may be unknown risks, discomforts or side effects

The less common risks and discomforts expected in this study are:

Risks associated with the psychophysiological procedure: You may become tired from sitting in front of the computer, or may become uncomfortable sitting in one position for a long period of time. You can take a break at any point during these tasks and you can stop at any point during the visit.

Risks associated with the saliva collection: You may feel uncomfortable producing saliva and spiting into the collection tube. You are free to take as long as you need for this process, you can take a break at any point, or discontinue the process if you wish.

Table 2 -The most common treatment-emergent adverse reactions (TEAR) considered possibly related to study medication occurring in study 401 and for which the incidence was ≥ 1%

System Organ Class	Preferred Term	Studies 401 (N=414) %
Gastrointestinal Disorders	Nausea	6
	Stomach discomfort	3
	Diarrhea	2
	Dyspepsia	1
	Constipation	1
	Hypoaesthesia oral	1
General Disorders and Administration Site Conditions	Fatigue	4
Nervous System Disorder	Dizziness	4
	Headache	1
	Dysgeusia	1
Psychiatric Disorders	Insomnia	1
Skin and Subcutaneous Tissue Disorders	Rash	1
	Pruritus	1
Hepatobiliary Disorders*	Alanine aminotransferase (ALT/SGPT) abnormal	1

* Alert terms (ALT and/or AST values >10 times the upper limit of normal) or abnormal values that led to study drug termination

At screening, study clinicians will discuss benefits and risks of study participation. Alternatives include not participating in the proposed study.

Adequacy of Protection Against Risks

Protection Against Risk:

Every effort will be taken to prevent injury or distress that may result from this study. Care will be taken to avoid bringing about undue psychological distress during the self-reported questionnaires. In the event that a subject becomes unduly distressed or scores >32 on the IDS-SR (indicating moderate to severe depressive symptoms) or endorses a score >2 on the suicide item of the IDS-SR (Question 18) or a score ≥ 3 on the PHQ-9 suicide item, the PI or his designee will be immediately contacted, and an appropriate clinical intervention plan will be developed. Emory has inpatient, outpatient and emergency room psychiatric services at all recruitment and evaluation sites. In addition, the PI Dr. Andrew H. Miller is a Board-certified psychiatrist and has extensive experience in administering standardized clinical assessments and handling psychiatric emergencies. As for blood drawing, standard sterile procedure for blood withdrawal will be used. Blood draws will be conducted by trained phlebotomists with

significant experience in the technique. In addition, the volume of blood withdrawn for this study will not exceed 40 mL over the course of the study (including screening and assessment visits).To minimize discomfort during the MRS scanning, patients will be provided with a head cushion and earplugs. Patients will also be informed about the progress of the procedure through a remote microphone. In the case of patients developing acute anxiety or panic, the scanning session will be terminated and patient provided enough support to cope with the feelings induced by the scanner. If a patient is uncomfortable with the skin cleansing during the psychophysiological task, it will be omitted. Patients will be told about the airblast prior to beginning the task. If they are uncomfortable with this or any part of the task, the task will be stopped. To reduce the likelihood of adverse reactions to riluzole, all patients will have a thorough history of allergic reactions reviewed and their first dose of riluzole will be administered under supervision of a study physician or nurse practitioner. In addition, all patients will be screened for evidence of liver disease including a screen for liver transaminases as well as hepatitis B and C and HIV. Liver transaminases will also be monitored at weeks 4 and 8, and subjects will be instructed to inform staff of any gastrointestinal symptoms.

Data and Safety Monitoring Plan (DSMP)

Although no high risk procedures are being conducted as part of this study, we will be following patients on an FDA approved study medication for 8 weeks. For this reason, we have elected to utilize the Data Safety Monitoring Board of the Department of Psychiatry and Behavioral Sciences as a third-party oversight committee. The DSMB is described in detail below. In addition, study clinicians will be available by pager 24 hrs/7 days a week during the period between screening and study completion. Should a subject experience a study related emergency or should active suicidal ideation develop, either Dr. Miller or Haroon will be immediately notified. Drs. Haroon and Miller are board certified psychiatrists with extensive experience in the treatment of psychiatric emergencies. If the assessment in question was done by a clinician other than either of them, one of them will immediately contact the subject and will evaluate the need for further medical or psychiatric treatment and will arrange medical or psychiatric follow-up if indicated. They will evaluate each case individually to make a determination regarding whether the subject can remain in the study or whether the subject should be terminated in addition to receiving a mental health referral.

Adverse Event Reporting

Enrolled participants will be monitored closely by study clinicians for any adverse events. All adverse events will be collected using the NCI Common Terminology Criteria for Adverse Events version 4.0. If any overt study-related adverse events occur, a decision will be made about study continuation. Additionally, a record of adverse events for study participants will be reported to the DSMC on a regular basis. Subjects will be closely monitored during the course of the study for development of any serious or unexpected adverse reactions. Those events meeting Emory IRB criteria for a reportable event will be reported to the IRB or the Winship Cancer Institute DSMC according to standard regulations and procedures. The Emory IRB Reportable Events Guidelines are included in an attachment.

Stopping rules

Individuals who exhibit significant suicidal ideation as indicated above will be discontinued from the study. Individuals who exhibit any evidence of an allergic reaction including rash, hives or difficulty breathing and individuals who exhibit more than a two-fold elevation in liver transaminases will be discontinued from the study and followed until the allergic reaction

resolves or the transaminases return to baseline. Individuals who exhibit suicidal ideation or a serious adverse reaction to riluzole will be unblinded by the study pharmacist, and clinical management will be supervised by an unblinded study physician. Patients who have taken less than 75% of their medication may be excluded from study participation. If study removal occurs at or after the 4 week study visit, the patient will undergo exit safety laboratory testing and physical examination. Urine drug tests will be conducted at each visit (except week 2) using test kits. Individuals who test positive for drugs of abuse may be terminated at the discretion of the PI.

Composition of the Data Safety Monitoring Board (DSMB)

Frequency of DSMB review for this protocol will follow recommendations from the IRB based on the assessed risk status of the study. The DSMB for this study will consist of the Clinical Research Oversight Committee with members including Boadie Dunlop, M.D. Chairman, Larry Tune M.D., Marian Evatt, MD and Tanja Mletzco MA. They have agreed to serve as the external DSMB for investigator initiated clinical trials conducted by Emory researchers in the Department of Psychiatry & Behavioral Sciences. If the DSMB requires additional specialized expertise to evaluate safety issues related to the performance of this study, a relevant specialist will be consulted by the DSMB.

Procedures and Responsibilities of the DSMB

The DSMB will meet monthly. This protocol will be submitted to the DSMB simultaneously with the initial submission to the Emory IRB. The DSMB will review the research protocol and plans for data and safety monitoring. Once per year (or after 6 months if the protocol is considered 'high risk' by the IRB), the DSMB will review a report from the study's data manager that includes: the number of participants who signed consent for the study and were randomized, the number of post-randomization dropouts, reasons for these dropouts, and any safety concerns, adverse events, an up-to-date consent form, and measures taken to protect confidentiality (e.g., data and tape storage, use of coded ID numbers, etc.). The DSMB will also review the Principal Investigator's summary of any new data or evidence that might alter the risk/benefit ratio for participating in the study (e.g., newly published studies, etc.). After reviewing this information, the DSMB will issue its own report summarizing any serious and unexpected adverse events or other unanticipated problems that involve risk to study participants, and whether these appear related to the study-based interventions or research assessment protocols.

There will be regular, ongoing communication between the PI, Emory's IRB, and the DSMB. The PI will take responsibility for submitting reportable serious and unexpected adverse events or other unanticipated study problems to Emory's IRB according to standard regulations. A copy will be sent to the DSMB. Actions taken by the IRB in response to adverse event reports will be immediately reported to the DSMB.

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