**SAFETY AND NEUROPROTECTIVE EFFECTS OF POLYPHENON E IN MULTIPLE SCLEROSIS (Phase II)**

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**SAFETY AND NEUROPROTECTIVE EFFECTS OF POLYPHENON E IN MULTIPLE SCLEROSIS**

**SCHEMA Pilot safety study of Polyphenon E in Subjects with Multiple Sclerosis (MS)**

Subjects 18-60 years of age with remitting/relapsing or secondary progressive multiple sclerosis (MS) and expanded disability status scale (EDSS) score of ?=7.0 who have been on stable therapy with Copaxone 20 mg, Rebif 44 mcg three times a week, Betaseron 250 mcg every other day or Avonex 30 mcg for at least six months or on no disease-modifying therapy for at least six months (n=10))

**Screening/Baseline Data Collection (visit 0 day -14):**

Sign informed consent, chart review, medical history, height, weight, vital signs, physical exam, interview for medications/supplements and current tea intake, EDSS, MS functional composite (MSFC), comprehensive metabolic panel, CBC with platelets, liver panel,pregnancy test (for women of child-bearing potential).

**Baseline Imaging (visit1, day 0):**

Pre-treatment Magnetic Resonance Imaging (MRI) with gadolinium

**Baseline Cognitive Testing and Quality of Life Assesment (visit 2, day 0-7):**

Multiple Sclerosis Functional Composite (MSFC), Modified Fatigue Impact Scale (MFIS), Medical Outcomes Survey Short Form 36 (SF-36), Beck Depression Inventory-II (BDI-II), Perceived Deficits Questionnaire (PDQ), MS Neuropsychological Screening Questionnaire (MSNQ), cognitive test battery,

Polyphenon E 400 mg EGCG bid or placebo for 6 months open label (n=10)

**Monitoring visit Peak and trough levels of EGCG (visit 3, month 1):**

Subjects will have a standardized breakfast and will be supervised taking their morning dose of Polyphenon E. Blood will be drawn for serum EGCG levels at 3 and 8 hours.

Liver panel; adverse events (AE’s); concomitant medications/supplements/tea intake; physical exam if warranted by AE’s.

**Monitoring visit (months 2-5; visits 4-7):**

Liver panel; adverse events (AE’s); concomitant medications/supplements/tea intake; physical exam if warranted by AE’s.

Additional tests at specified monitoring visits: CMP and CBC with platelets (months 2, 3); physical exam, vital signs, weight/height, drug compliance/dispensing (month 3).

**Exit clinical and cognitive evaluation (month 6, visit 8)**

Cognitive testing, EDSS, MSFC; SF36, PDQ, MSNQ, MFIS and BDI-II

Adverse event evaluation;

physical exam, vital signs, weight/height;

EDSS, MSFC;

SF36, PDQ, MSNQ, MFIS and BDI-II;

**Exit imaging visit (month 6, visit 9)**

End of treatment Magnetic Resonance Imaging (MRI) with gadolinium

cognitive testing at 24 months.

Endpoints:

Safety:

Frequency and severity of adverse events

Efficacy:

Exploratory endpoints:

Change of brain N-acetyl-aspartate (NAA) levels over 6 months;

Change of NAA in chronic lesions, active lesions, and normal appearing white and normal appearing white gray matter;

Change of peak T1 times;

Change in cognitive performance;

Number of combined unique active lesions

Progression of disability (EDSS score, MSFC score);

Quality of life (SF-36,PDQ, MFIS, BDI-II)

**SCHEMA Phase II Randomized, Placebo-controlled Trial of Polyphenon E in Subjects with Multiple Sclerosis**

Subjects 18-60 years of age with remitting/relapsing or secondary progressive multiple sclerosis (MS) and expanded disability status scale (EDSS) score of (EDSS) score of≤ 7.0 who have been on stable therapy with Copaxone 20 mg, Rebif 44 mcg three times a week, Betaseron 250 mcg every other day or Avonex 30 mcg for at least six months (n=48)

**Screening/Baseline Data Collection (visit 0 day -14):**

Sign informed consent, chart review, medical history, height, weight, vital signs, physical exam, interview for medications/supplements and current tea intake, EDSS, MS functional composite (MSFC), comprehensive metabolic panel, CBC with platelets, liver panel,pregnancy test (for women of child-bearing potential)

**Baseline Cognitive Testing, Quality of Life Assesment, and Smell Test (visit 1, day 0-7):**

Multiple Sclerosis Functional Composite (MSFC), Modified Fatigue Impact Scale (MFIS), Medical Outcomes Survey Short Form 36 (SF-36), Beck Depression Inventory-II (BDI-II), Perceived Deficits Questionnaire (PDQ), MS Neuropsychological Screening Questionnaire (MSNQ), cognitive test battery, Smell Test

**Baseline Imaging (visit 2, day 0-7):**

Pre-treatment Magnetic Resonance Imaging (MRI) with gadolinium

Randomization to Polyphenon E 400 mg EGCG bid or placebo for one year (visit 2, day 0-7) (n=24 per group)

**Monitoring visit Peak and trough levels of EGCG (visit 3, month 1):**

Subjects will have a standardized breakfast and will be supervised taking their morning dose of Polyphenon E. Blood will be drawn for serum EGCG levels at 3 and 8 hours.

Liver panel; adverse events (AE’s); concomitant medications/supplements/tea intake; physical exam if warranted by AE’s

**Monitoring visit (months 2-11 ; visits 4-14):**

Liver panel; adverse events (AE’s); concomitant medications/supplements/tea intake; physical exam if warranted by AE’s.

Additional tests at specified monitoring visits: CMP and CBC with platelets (months 2, 3, 6, 12); physical exam, vital signs, weight/height, drug compliance/dispensing (every third month);

Smell tests to ensure no loss of olfactory senses or function every third month (months 3, 6, 9, 12);

EDSS, MSFC (every six months); SF36, PDQ, MSNQ, MFIS and BDI-II (at 12 months).

**Exit clinical and cognitive evaluation. (month 12, visit 15)**

Adverse event evaluation;

Smell Test;

physical exam, vital signs, weight/height;

EDSS, MSFC;

SF36, PDQ, MSNQ, MFIS and BDI-II;

cognitive testing at 12 months.

**Exit imaging visit (month 12, visit 16)**

End of treatment Magnetic Resonance Imaging (MRI) with gadolinium

**Post-treatment Follow-up Visit (month 13, visit 17):**

Telephone contact one month after discontinuing treatment to assess adverse events.

**Endpoints:**

**Safety:**

Frequency and severity of adverse events

**Efficacy:**

Primary endpoint: Difference in the rate of change of brain N-acetyl-aspartate (NAA) levels;

Secondary endpoints:

* Difference in the rate of change of NAA in chronic lesions, active lesions, and normal appearing white and normal appearing white gray matter;
* Difference in the rate of change of peak T1 times;
* Difference in the rate of brain atrophy;
* Difference in cognitive performance;
* Number of relapses
* Number of combined unique active lesions
* Progression of disability (EDSS score, MSFC score);
* Quality of life (SF-36,PDQ, MFIS, BDI-II)

# APPENDICES

Appendix A:McDonald Criteria for Multiple Sclerosis Diagnosis

Appendix B: Lublin and Reingold Classification for Relapsing/Remitting Multiple Sclerosis

Appendix C: Expanded Disability Status Scale (EDSS) Score

Appendix D: Data safety and monitoring board charter and data safety and monitoring plan

Appendix E: Product information submitted to NIH

Appendix F: Investigators brochure

Appendix G: Questionnaires BDI, PDQ, MSNQ, MFIS, MSFC, SF-36

# OVERALL RESEARCH PLAN

This protocol consists of an initial pilot study and a phase II study. Our completed pilot study provided preliminary evidence of safety of Polyphenon E in subjects with MS as well as preliminary evidence for a neuroprotective effect. This safety evidence along with safety data from completed studies with Polyphenon E in patients with Chronic Lymphocytic Leukemia.

# OBJECTIVES

## Pilot study

**Primary Objectives:**

Determine the safety of Polyphenon E, 400mg EGCG twice a day for six months in subjects with MS.

**Secondary Objectives**

Obtain preliminary imaging and clinical data on treatment of subjects with MS with Polyphenon

## Phase II study

The goal of this investigation is to evaluate the safety and efficacy of Polyphenon E in patients with relapsing/remitting multiple sclerosis (MS) or secondary progressive MS and an Expanded Disability Status Scale (EDSS) score of up to 7.0 who have been on stable therapy with Copaxone 20 mg, Rebif 44 mcg three times a week, Betaseron 250 mcg every other day or Avonex 30 mcg for at least six. Specific objectives are listed below.

**Primary Objectives:**

Determine if treatment with Polyphenon E, 400 mg EGCG twice a day for one year alters the rate of change of brain N-acetyl-aspartate (NAA) levels in subjects with MS compared with those taking placebo.

Determine the safety of treatment with Polyphenon E at a dose containing 400 mg of EGCG twice a day over one year in subjects with MS.

**Secondary Objectives:**

Determine if treatment with Polyphenon E alters the rate of change of brain atrophy and peak T1 times, two additional measures of tissue integrity.

Compare the changes in number of new T2 lesions on MRI in MS patients treated with Polyphenon E vs. placebo.

Compare cognitive decay in subject with MS treated with Polyphenon E vs. placebo

Evaluate changes in disease activity, progression of disability and quality of life in remitting/relapsing MS patients treated with Polyphenon E vs. placebo.

**Exploratory Objectives:**

Correlate the peak and trough plasma EGCG levels with the changes in NAA levels.

Determine the effects of treatment with Polyphenon E on the rate of change of NAA levels in normal-appearing white matter, grey matter, active lesions and chronic lesions.

# BACKGROUND

**Multiple Sclerosis (MS)**

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system. Up to 70% of people with multiple sclerosis will experience increasing disability over time.

**The therapies for MS currently available have side effects and are only partially effective in slowing progression of disability.**

These therapies include interferon β-1abeta-1a (Avonex®, Rebif®) , interferon β-1b(Betaseron®) , glatiramer-acetate (Copaxone®), mitoxantrone (Novantrone®) and Natalizumab (Tysabri®). All of the currently available therapies target inflammation by different mechanisms. Interferon β interferes with the migration of inflammatory cells across the blood brain barrier, down-regulates antigen presentation in the CNS and induces TH-2 cells that regulate inflammation in the brain. Glatiramer-acetate induces TH-2 cells that cross into the CNS and regulate inflammation. Mitoxantrone is cytotoxic chemotherapy agent that has widespread immunosuppressive activity over T and B lymphocytes as well as macrophages. Natalizumab is a monoclonal antibody against α-4 integrin. Alpha 4 integrin is an adhesion molecule expressed by activated lymphocytes and is essential for their migration into the CNS.

Interferon β is effective at reducing relapses. The higher doses (Rebif®, Betaseron®) may be more effective than the lower dose (Avonex®). Common side effects of interferon β frequently are flu like symptoms and depression. Other serious but rare side effects include possible liver injury and decreased platelet counts. The systemic symptoms are bothersome for many patients and are more pronounced when using the higher doses. Higher doses of interferon are also more likely to induce antibodies that interfere with the activity of interferon. Glatiramer acetate does not have mayor systemic side effects but its effect of action is slower than interferon β. Glatiramer acetate and the different forms of interferon β are considered first line treatments for MS because of their proved safety record. The frequency of relapses is reduced by about one third with either interferon or glatiramer acetate. Many patients continue to have persistent disease activity when treated with either interferon or copaxone and the effect of either of these two therapies on the progression of disability is incomplete.

Mitoxantrone is a more toxic drug. It can cause multiple systemic side effects, many of them serious. Serious systemic side effects include neutropenia, immunosuppression, ovarian failure and heart failure. Cardiotoxicity can occur at any dose but is more frequent after a cumulative dose above 100mg/meter2 . Cardiotoxicity limits the length of treatment to 2 years. . Because of its toxicity, mitoxantrone is usually used as a second line agent after people fail the first line therapies.

Natalizumab (Tysabri®) is the most recent addition to the treatment alternatives for MS. Natalizumab is very effective at decreasing the number of new lesions on MRI, decreasing the frequency of relapses and slowing disease progression. Natalizumab in combination with Avonex® is known to be more effective than Avonex® alone. Because of natalizumab mechanism of action is so selective it has very few systemic side effects. The enthusiasm about this new drug was curbed when cases of progressive multifocal leukoencephalopathy (PML) were seen after the completion of the initial Phase-III trials. PML is a serious viral infection of the brain. The only current treatment available is stopping natalizumab to allow the immune system to control the infection. PML is usually fatal and when it isn’t fatal it usually results in severe disability. Natalizumab is now reserved for patients that fail the first line therapies.

**Neuroprotective therapies for MS would be a very valuable treatment for people with MS.**

**Recent pathologic studies suggest that neurodegenerative mechanisms may be the main cause of axonal loss in MS. Axonal injury occurs frequently even at early stages of MS and is believed to be the mechanism for irreversible progression of disability. Axonal loss occurs in the relapsing phase of the disease where inflammation is prominent and continues at a higher rate when the disease enters a progressive phase. In the progressive phases of the disease or when MS starts with progression of disability since its onset, inflammation is less evident. All of the current therapies protect axons indirectly by targeting inflammation, the cause of acute axonal damage, but do not target directly the mechanisms in the axon that lead to its slow death in the progressive phases of the disease. Treatments that directly protect axons could thus have a great impact in slowing the progression of disability in people with MS.**

**Advanced MRI techniques provide measures that can evaluate the effectiveness of neuroprotective therapies prior to phase III studies.**

MRI has provided new insights into the pathogenesis of MS and has proven to be a tremendous tool in assessing the effectiveness of new therapies for MS. All of the FDA approved anti-inflammatory therapies for MS have positive effects on disease activity assessed by brain MRI. These effects include decreased number of gadolinium-positive and new T2 lesions and slowing the rate of increase in of volume of the T2 lesion burden. Importantly, the ability to indirectly measure new inflammatory lesions by measurement of gadolinium enhancement and new T2 lesions has greatly facilitated testing of anti-inflammatory therapies in MS. Indeed, Phase II studies of new anti-inflammatory therapies typically will use number of gadolinium enhancing lesions as a sensitive primary outcome measure. However there is uncertainty about how best to assess neuroprotective therapies using MR technologies. Three approaches appear promising: measuring NAA by MRS, quantitative T1 mapping, and determining the rate of brain atrophy. Each of these techniques has its own strengths and weaknesses, and by utilizing them together, it may be possible to create a more comprehensive picture of the degenerative process in MS.

NAA is synthesized from aspartate and acetyl-coenzyme A in neurons. In early postnatal life NAA participates in lipid and steroid synthesis pathways that are basic for myelin synthesis. Later in life NAA appears to play other roles besides myelin turnover including an important bioenergetic role in neuronal mitochondria. The production of NAA may facilitate the removal of aspartate from neuronal mitochondria, thus favoring conversion of glutamate to alpha ketoglutarate which can enter the tricarboxylic acid cycle for energy production. Histopathological studies have shown that NAA is a selective marker of neurons and axons. NAA can be measured by MRS and is thus a key imaging marker of neuronal health, viability and number. Initial studies of NAA were limited to a single region of interest but new techniques allow for simultaneous acquisition of multiple voxels. These spectroscopic imaging techniques can provide maps of NAA throughout the brain. These NAA maps can be registered to segmented maps of high resolution conventional MRI images, allowing the determination of NAA in white matter and grey matter lesions. Recent advances in the techniques for MRS may allow faster acquisition times and permit wider tissue coverage without excessive scanning time.

Quantitative T1, is an imaging technique that is very sensitive to subtle tissue damage and provides a good overall summary of brain health. T1 is a fundamental time constant in magnetic resonance that provides a measure of how quickly longitudinal magnetization returns to equilibrium following a perturbation. This T1 value determines the degree of magnetization saturation at times following a radiofrequency pulse, and is a principal source of contrast in MRI. T1 values can be measured precisely with excellent spatial resolution and in reasonable imaging times on the current generation of MR scanners. T1 is determined by factors related to the local tissue chemistry including the macromolecular content, concentration of paramagnetic compounds, temperature, and pH. The main determinant of brain tissue 1H2O T1 values is the content of macromolecules. Because axons and myelin are rich in macromolecules, axonal and myelin loss will cause changes in T1 values. T1 values have been shown to correlate with histopathological measures of myelin content and also although less strongly with axonal loss. Analysis of whole-brain T1 histograms provides an efficient summary of total tissue damage, including changes in the normal appearing white matter. The quantitative T1 measurements are highly correlated to the results of magnetization transfer ratio (MTR) imaging, another imaging technique that is being widely used in MS studies because of its sensitivity to tissue damage. However, the T1 histograms have the advantage over MTR histograms of having two well-demarcated peaks corresponding to grey and white matter 1H2O that allow rapid unsupervised image segmentation.

Brain atrophy is another useful imaging technique to monitor neurodegeneration. Several automated approaches are available and provide reliable reproducible estimates of tissue loss. Atrophy has been shown to be sensitive to the treatment effects of interferon-βinterferon-b in patients relapsing remitting MS (RRMS) and with CIS. Although atrophy measures are not specific for axonal loss and may be confounded by changes in inflammatory activity, the validity of atrophy as a surrogate marker has been partially established in longitudinal studies where the rate of development of atrophy predicts higher rates of progression of disability and cognitive impairment.

**Polyphenon E**

Polyphenon E is a botanical drug substance manufactured by Mitsui Norin Co., Ltd. (Mitsui Norin; Shizuoka, Japan); it contains a mixture of catechins originating from the leaves of green tea (*Camellia sinensis*). Several clinical investigations are currently underway with the goal of demonstrating the efficacy and safety of Polyphenon E in a variety of indications and patient populations. Several studies evaluating cancer chemopreventive efficacy are being conducted under an IND sponsored by the National Cancer Institute, Division of Cancer Prevention (NCI, DCP). The Polyphenon E drug product that will be used in the proposed investigation is a hard gelatin capsule standardized to contain 200 mg epigallocatechin gallate (EGCG) per capsule; the drug product capsules are manufactured under a contract between Mitsui Norin and NCI, DCP and supplied through an agreement between Polyphenon E International, Inc. (New York, NY) and NCI, DCP. NCI, DCP has provided a letter permitting FDA to reference the NCI, DCP-sponsored IND for safety and chemistry information relevant to the review of the current application.

Polyphenon E contains 85–95% total catechins by weight; the main component is EGCG, which comprises 56–72% of the material. Other catechins present in Polyphenon E include epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), gallocatechin gallate (GCG), gallocatechin (GC), catechin gallate (CG), and catechin. NCI, DCP has sponsored four completed Phase I studies with Polyphenon E and/or EGCG. These include single- and multidose safety and pharmacokinetic (PK) studies with oral Polyphenon E and EGCG; safety and PK of Polyphenon E administered in a fed *vs*. fasted state; and a drug interaction study with Polyphenon E. Doses of Polyphenon E in these studies ranged from 200 mg EGCG to 1200 mg EGCG per day, with administration periods of up to four weeks. Briefly, results indicate that both formulations are generally well tolerated; the most common adverse events were mild gastrointestinal complaints, more common with increasing dose and under fasting conditions. Some subjects also experienced headaches and fatigue, possibly related to study products, but not clearly associated with dose level. Plasma EGCG levels increased with dose, and formulation had no effect on EGCG PK. Several studies with longer administration periods are currently underway.

The safety of tea and tea compounds is presumably supported by centuries of consumption by the human population. However, in recent years, oral use of green tea extracts (GTEs) has been associated with several instances of hepatotoxicity. Therefore, clinical studies with Polyphenon E require adequate safety monitoring of subjects, with particular attention to effects on liver function. Minimization of potential harmful effects on the liver is managed by delivering the dose with food, and performing frequent liver function tests (LFTs). In this study, liver function tests will be performed prior to initiating treatment with study drug, and monthly thereafter.

**Rationale**

The health benefits associated with consumption of tea, particularly green tea, has become an area of intense interest and investigation. Green tea and its constituent catechins are best known for their antioxidant properties, which has led to their evaluation in a number of diseases associated with reactive oxygen species (ROS), such as cancer, cardiovascular and neurogenerative diseases (reviewed in ).

EGCG been shown to effectively treat experimental autoimmune encephalitis (EAE), the animal model of MS, in SJL mice. Drs. Gail Marracci and Dennis Bourdette, from the group Dr.Lovera was a part of when he was at OHSU, have replicated these results in the EAE model. Drs. Marracci and Bourdette have also shown that low doses of 475mg/kg/day have a purely neuroprotective effect on the axons from animals with EAE (Figure 1).

A recent pharmacokinetic study in mice shows that a dose of 500 mg EGCG/kg-bw/day results in a total serum level EGCG of 1 μg/ml and in free EGCG levels of 0.1 to 0.55 μg/ml. These levels are similar to the free EGCG levels (0.29 ± 0.124 μg/ml) that are seen in humans after repeated dosing with Polyphenon E at a dose of 800 mg of EGCG per day. Therefore, the serum EGCG levels effective for protection against axonal loss can be achieved in humans at Polyphenon E doses that have been shown to be safe.

There have been no studies beyond our pilot study evaluating the effect of Polyphenon E or other green tea extracts in subjects with MS. The current Phase II trials of disease modifying agents in MS have used enhancing lesions as a marker of disease activity. However, evaluating neuroprotective therapies in early trial in MS is challenging because the usual MRI measurements do not reflect axonal loss. We have selected the change in NAA levels as the main outcome because they are a selective measure of axonal number and function. We have included additional advanced imaging techniques that we expect will be sensitive to the effects of a neuroprotective agent as secondary outcomes. We have measures of disability progression, cognitive function and quality of life. We expect that this data from the clinical measures will help in the design of future studies.

We expect that our study will provide sufficient evidence that Polyphenon E is a safe and effective neuroprotective agent and justify continued development for this indication.

This research is significant because it will result in a new treatment that slows the progression of disability beyond what current therapies offer. Such a treatment will improve the quality of life of people with MS, reduce their economic losses due to reduced working capacity, and reduce the costs related to long-term care and hospitalization. Finally, the benefits of a safe neuroprotective agent could extend to other neurodegenerative diseases, such as Alzheimer’s and Parkinson’s disease.

# SUMMARY OF STUDY PLAN

We have completed a pilot study with 10 subjects. Now we propose a Phase II randomized, placebo-controlled trial to confirm the preliminary results seen in the pilot study.

## Pilot safety study

The pilot phase of the study will be an open label 6 month study. The inclusion criteria will be the same as the criteria used for the Phase II study. We will evaluate the subjects with the same outcome measures and procedures as described below for the full Phase II. All subjects will be treated with Polyphenon E (400 mg EGCG twice a day) for six months. The pilot study will also be monitored by the DSMB. After completion of this pilot phase we will reapply to the FDA with the safety data from this pilot phase and obtain authorization for the Phase II study.

The main outcome of this pilot phase will be safety. The cognitive and MRI data will be analyzed as exploratory outcomes.

## Phase II study

The second phase to be conducted after the completion of the pilot study will be a Phase II randomized, placebo-controlled trial in subjects with remitting/relapsing or secondary progressive MS who are taking Copaxone and have been on stable treatment with Copaxone for at least six months, or are on no therapy for six months and are refusing therapy and who have not relapsed within 30 days prior to enrolling in the study. Forty-eight subjects will be accrued, 24 per arm, with the goal completing 19 evaluable subjects per arm. Screening tests will include a physical exam and blood chemistries.

Baseline assessment will include the MS functional composite (MSFC), the expanded disability status scale (EDSS) assessment, and questionnaires including the Medical Outcomes Survey Short Form 36(SF-36), the Modified Fatigue Impact Scale, Beck Depression Inventory-II (BDI-II), the Perceived Deficits Questionnaire (PDQ), the Multiple Sclerosis Neropsychological Screening Questionnaire (MSNQ) and a cognitive battery. The cognitive battery will consist of the following tests: the Paced Auditory Serial Addition Task (PASAT), the Controlled Word Association Test (COWAT), the California Verbal Learning Test-II (CVLT II) and the Victoria version of the Stroop test. Eligible subjects will have a baseline MRI with gadolinium. If the subject able to complete the scan he or she will be randomized to receive Polyphenon E (400 mg EGCG bid) or placebo (two capsules twice a day) for two years.

Subjects will return for monthly visits until the end of the study. Blood collection, symptom assessments and concomitant medication review will be performed at each clinic visit. Peak and trough levels of EGCG will be measured after one month on the full dose of Polyphenon E.

Clinic visits at three-month intervals will also include a general physical exam and compliance assessment (pill count); additional study drug will also be dispensed at these visits. The EDSS and MSFC will be repeated every six months; MRI, clinical assessment, SF-36, PDQ, MSNQ, MFIS and BDI-II will be repeated at six months, one year, and one year visits. The cognitive battery will be performed at baseline and at one year.

Study endpoints include comparison between groups in: the rate of change with treatment in NAA levels in the brain; rate of change of brain atrophy and quantitative T1; new lesion numbers; number of relapses; percentage of subjects with sustained progression; changes in SF-36, PDQ, MSNQ, MFIS; cognitive changes and safety.

Assuming a screening rate of approximately 6 participants per month and an accrual rate limited to approximately 3 participants per month, we expect accrual to be complete in 15 months. The entire study will be completed over a period of approximately four years; each subject will participate for approximately 13 months (12 months of treatment and one month of follow-up)*.*

# PARTICIPANT SELECTION

## Inclusion Criteria

Diagnosis of MS by McDonald criteria (Appendix A).

Relapsing-remitting MS or secondary progressive MS according to Lublin and Reingold classification (Appendix B) .

Stable therapy with Copaxone 20 mg, Rebif 44 mcg three times a week, Betaseron 250 mcg every other day or Avonex 30 mcg for at least six months.

EDSS Score less than or equal to 7.0 (Appendix C).

Ages 18-60. No dosing or adverse event data are currently available on the use Polyphenon Ein participants <18 years of age. Children <18 are excluded from this study but will be eligible for future pediatric trials, if applicable.

Participants must have normal organ and marrow function as defined below:

|  |  |
| --- | --- |
| a) Leukocytes | ≥3,000/µL |
| b) Absolute neutrophil count | ≥1,500/µL |
| c) Platelets | ≥100,000/µL |
| d) Total bilirubin | ≤local upper limit of normal |
| e) AST (SGOT) | ≤local upper limit of normal |
| f) ALT (SGPT) | ≤local upper limit of normal |
| g) Creatinine | ≤local upper limit of normal |

The effects of Polyphenon E on the developing human fetus at the recommended therapeutic dose are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her study physician immediately.

Ability to understand and the willingness to sign a written informed consent document.

Willing to drink at most one cup of black tea and two cups of coffee per day, and abstain from drinking green tea or taking supplements containing green tea or green tea compounds, for the duration of the investigation.

## Exclusion Criteria

MS relapse within the 30 days prior to enrollment.

A primary progressive form of MS.

Previous treatment prior to study entry as follows: complete radiation ablation of the bone marrow or anti-CD4 antibody treatment (Campath) at any time; mitoxantrone, cyclophosphamide, 11nblended11ne, Natalizumab or other immunomodulatory or immunosuppressant therapies except for Copaxone or methylprednisone for relapses within prior nine months.

History of renal or liver disease.

Consumption of green tea or supplements containing green tea or tea extract within 30 days prior to enrollment.

Participants may not participate in any other clinical trial involving investigational agents during the study, or within six months prior to enrolling in the study.

History of allergic reactions attributed to compounds of similar chemical or biologic composition to Polyphenon E, tea, or any of the inactive ingredients present in the active or placebo capsules, including gelatin.

History of allergic reactions to gadolinium or any other condition contraindicated for MRI.

Uncontrolled, clinically-relevant active illness (aside from MS) including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

Any condition which would make the subject, in the opinion of the investigator, unsuitable for the study.

Inability to complete the baseline MRI scan.

Pregnant women are excluded from this study because Polyphenon E is an Investigational agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with Polyphenon E, breastfeeding should be discontinued if the mother is treated with Polyphenon E.

Any underlying predisposition to gastrointestinal bleeding (peptic ulcer disease, gastritis, diverticulitis, colitis, hemorrhoids).

## Description of Study Population

### Pilot study

Both men and women and members of all races and ethnic groups are eligible for this trial.

|  |  |  |  |
| --- | --- | --- | --- |
| TARGETED/PLANNED ENROLLMENT: | | | |
| Ethnic Category | Sex/Gender | | |
|  | Females | Males | Total |
| Hispanic or Latino | 0 | 0 | 0 |
| Not Hispanic or Latino | 8 | 2 | 10 |
| Ethnic Category: Total of All Subjects \* | 8 | 2 | 10 |
| Racial Categories |  | | |
| American Indian/Alaska Native | 0 | 0 | 0 |
| Asian | 0 | 0 | 0 |
| Native Hawaiian or Other Pacific Islander | 0 | 0 | 0 |
| Black or African American | 2 | 0 | 2 |
| White | 6 | 2 | 8 |
| Racial Categories: Total of All Subjects \* | 8 | 2 | 10 |
| The “Ethnic Category: Total of All Subjects” must be equal to the “Racial Categories: Total of All Subjects.” | | | |

### Phase II study

|  |  |  |  |
| --- | --- | --- | --- |
| TARGETED/PLANNED ENROLLMENT: | | | |
| Ethnic Category | Sex/Gender | | |
|  | Females | Males | Total |
| Hispanic or Latino | 2 | 0 | 2 |
| Not Hispanic or Latino | 35 | 11 | 46 |
| Ethnic Category: Total of All Subjects \* | 37 | 11 | 48 |
| Racial Categories |  | | |
| American Indian/Alaska Native | 0 | 0 | 0 |
| Asian | 1 | 0 | 1 |
| Native Hawaiian or Other Pacific Islander | 0 | 0 | 0 |
| Black or African American | 7 | 2 | 9 |
| White | 29 | 9 | 38 |
| Racial Categories: Total of All Subjects \* | 37 | 11 | 48 |
| The “Ethnic Category: Total of All Subjects” must be equal to the “Racial Categories: Total of All Subjects.” | | | |

**Recruitment and Retention Plan**

Subjects will be recruited from the LSU MS clinic and from the general public. Advertisements will be placed in the local newspapers as well as in the LSU clinical trials website. Advertisement fliers will be posted in the bulletin boards at LSU and in the waiting area of the MS clinic. Efforts to include minority groups will include advertisements in local foreign language newspapers that target Hispanics and Asians and advertisements on the bulletins of local churches and other organizations that are attended by a high percentage of minorities. We will also give brief presentations about the study to support groups around the city.

# AGENT ADMINISTRATION

Intervention will be administered on an outpatient basis. Study drug will be dispensed by the research coordinator at LSU.

Reported adverse events and potential risks are described in section .

**Dose Regimen and Dose Groups**

Pilot study

The subjects will receive Polyphenon E at a dose containing 400 mg of EGCG (two 200 mg capsules) twice daily.

Phase II

Eligible subjects will be randomized to receive Polyphenon E at a dose containing 400 mg of EGCG (two 200 mg capsules) twice daily, or matching placebo capsules twice daily. The administration period is one year.

**Study AgentAdministration**

* Subjects will self-administer the study drug.
* Capsules will be taken on a full stomach, within one hour of eating a substantial meal.

**Contraindications**

Polyphenon E is contraindicated in patients who are hypersensitive to tea products or any of the inactive ingredients found in the drug product capsules.

**Concomitant Medications**

There are no proscribed medications, but patients will be asked to refrain from drinking any form of green tea or consuming products containing green tea during the study. Concomitant medication information will be collected to assist in determining adverse event attribution.

All medications (prescription and over-the-counter), vitamin and mineral supplements, and/or herbs taken by the participant will be documented on the concomitant medication CRF and will include: 1) start and stop date, dose and route of administration, and indication. Medications taken for any procedure (*e.g.,* MRI) will also be recorded.

**Dose Modification**

Dose modifications are allowed and will be made as follows:

Grade 1 liver function test (LFT) elevation (>ULN - 2.5x ULN): Study drug will be withheld and repeat panels will be obtained at least weekly until resolution to normal; study drug will then be reintroduced at original level. If LFT elevation returns after study drug is reintroduced, study drug will be permanently discontinued, and repeat panels will be obtained at least weekly until resolution to normal

*Note: The LFT panel includes AST, ALT, ALP, albumin, total and direct bilirubin, and total protein)*

Grade 2 (2.5x ULN – 5.0x ULN), Grade 3 (5.0x ULN – 20.0x ULN), or Grade 4 (>20.0x ULN) LFT elevation: Study drug will be permanently discontinued and repeat panels will be obtained at least weekly until resolution to normal.

Other grade 2 or higher adverse events probably or definitely related to drug that do not resolve spontaneously within three days or do not respond to appropriate palliative care: Dose will be reduced by one half until symptoms resolve; study drug will then be reintroduced at the initial, prereduction level. If symptoms do not improve within three days at the reduced dose, or return at grade 2 or higher after resuming administration at the full dose level, study drug will be discontinued.

Dose will be reduced by one half if vomiting of the study drug occurs on two consecutive days; if vomiting persists at the reduced dose then the study drug will be discontinued.

**Adherence/Compliance**

Protocol adherence/compliance will be measured by pill counts at office visits as noted and at the end of the study. Pill counts will be entered in the Compliance Form. Compliance is defined as having taken 75% or more of the daily medications.

# PHARMACEUTICAL INFORMATION

**Polyphenon E**

Polyphenon E is a botanical drug substance containing a mixture of catechins originating from the leaves of green tea (*Camellia sinensis*). To manufacture Polyphenon E, a hot water extract of green tea is extracted further with ethyl acetate. The resulting crude extract is dissolved in methanol and purified by affinity column fractionation. Once dried, the final product contains 85–95% total catechins; the main component is EGCG, which comprises 56–72% of the material. Other catechins present in Polyphenon E include epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), gallocatechin gallate (GCG), gallocatechin (GC), catechin gallate (CG) and catechin. Polyphenon E contains minimal amounts of caffeine, (<1.0%) and may also contain small quantities of theobromine (<1.0%) and gallic acid (<0.5%).

The investigational product to be used in the proposed clinical investigation is a dark green, opaque, size 0 hard gelatin capsule containing enough Polyphenon E to deliver 200 mg EGCG per capsule. Polyphenon E Capsules are manufactured, stored, distributed, and evaluated for stability under contract to NCI, DCP using current good manufacturing procedures (cGMP) as outlined in the US Code of Federal Regulations. Inactive excipients in capsules include microcrystalline cellulose NF, cros­carmel­lose sodium NF, colloidal silicon dioxide NF, and magnesium stearate NF. Placebo capsules contain pregelatinized starch NF, microcrystalline cellulose NF, colloidal silicon dioxide NF, and magnesium stearate NF. Capsules are packaged in high-density polyethylene (HDPE) containers with child-resistant closures, and stored under controlled room temperature conditions.

Clinical studies investigating the safety and chemopreventive efficacy of Polyphenon E are being conducted under an IND sponsored by NCI, DCP and filed in FDA’s Division of Drug Oncology Products. NCI, DCP has provided a letter permitting FDA to refer to this IND for information relevant to the review of the current application.

**Reported Adverse Events and Potential Risks**

In an NCI, DCP-sponsored Phase I single-dose study, Polyphenon E and EGCG administered over the dose range of 200–800 mg EGCG were well tolerated by the 20 participating subjects. No serious adverse events were reported. The most frequently reported adverse events were headache and fatigue, which in some cases were possibly related to the study products. One case of grade 1 abdominal pain was considered possibly related to study drug, and one case of grade 1 dry mouth was reported with an unknown relationship to study drug. Other events were considered not related to study drug.

In a multidose Phase I study sponsored by NCI, DCP, 40 subjects completed four weeks of treatment with placebo capsules, or Polyphenon E or EGCG (both providing EGCG doses of 400 mg bid or 800 mg qd) . Adverse events were predominantly mild; no serious adverse events were reported. No events were considered possibly, probably, or definitely related to study drug treatment. In some cases, the relationship of adverse events to study drug was unknown (*i.e.,* flatulence, gas, and nose bleed). For most events, the incidences reported in the treatment groups were not more than those in the placebo group. More incidences of nausea were reported following 800 mg qd treatment with Polyphenon E or EGCG, and more incidences of abdominal pain were reported in the 800 mg EGCG qd group than in the other groups. Grade 2 events of allergic rhinitis and seasonal allergy were observed in both Polyphenon E and EGCG treatment arms; neither was related to the study drug treatment. Minor changes were observed in CBC and blood chemistry profiles after repeated administration of green tea polyphenol products.

An NCI, DCP-sponsored Phase I study in 30 healthy subjects was conducted to determine the effects of fasting on the pharmacokinetics of green tea catechins following a single oral dose of Polyphenon E . Subjects received two identical doses of Polyphenon E (400, 800, or 1200 mg EGCG), one in a fed and one in a fasting state, separated by a one-week wash-out period. Frequently reported adverse events included grade 1 and 2 nausea, headache, and abdominal pain. In some instances, these were possibly or probably related to study medication. Asthenia was also reported frequently, but was not related to study medication. Reports of nausea, and possibly abdominal pain, considered possibly or probably related to drug administration, appeared to increase in frequency with increasing dose, especially under fasting conditions. Other adverse events possibly or probably related to Polyphenon E administration did not have a clear dose-response relationship. Infrequent events that at times were considered possibly or probably related to study medication included dizziness, diarrhea, eructation, dyspepsia, chest pain, and rash. Gastrointestinal adverse events were usually mild and seen most often in the fasting condition and at the highest dose level. Onset of gastrointestinal events typically occurred within 2–3 hours of dosing and resolved within two hours. Headaches and fatigue were not dose-related and may have been related to abstinence from caffeine or other procedure-related stresses.

A fourth completed NCI, DCP-sponsored Phase I study with Polyphenon E was conducted to determine the effect of repeated administration of Polyphenon E on cytochrome P450 (CYP) and glutathione-*S*-transferase (GST) enzyme activities. Forty-two subjects took Polyphenon E, providing 800 mg EGCG qd, for four weeks. The most severe adverse events were grade 2 events of headache, rhinitis, nausea, arthralgia, myalgia, dyspepsia, and dysphagia. Frequently reported events included nausea, headache, abdominal pain, dyspepsia, and diarrhea. Events at times considered definitely, probably or possibly related to study drug included nausea, abdominal pain, diarrhea, dyspepsia, vasodilation, flatulence, dizziness, and vomiting. Polyphenon E had minimal effects on four major CYP isozymes; the only significant effect observed suggests a small reduction in CYP3A4 activity. However, significantly increased GST enzyme activity and GSTπ enzyme levels were seen in subjects with low baseline values. Results suggest that Polyphenon E administration is not likely to affect the PK of commonly used pharmaceutical drugs, but could enhance the detoxification of carcinogens in persons with low baseline GST activity/level.

Overall, Polyphenon E has been well tolerated in the four completed Phase I studies sponsored by NCI, DCP: Aes have generally been mild, with no serious Aes reported. Aes reported with a possible relationship to study drug include asthenia, headache, abdominal pain, chest pain, diarrhea, dyspepsia, eructation, flatulence, nausea, vomiting, dizziness, vasodilation, and rash. No events of grade 3 or higher were reported with a possible relationship to study drug. Grade 2 events reported with a possible relationship to study drug include asthenia, headache, abdominal pain, dyspepsia, nausea and rash. The most frequent events in completed studies that at times were considered drug-related include headache, nausea, abdominal pain, diarrhea, dyspepsia, dizziness, and asthenia.

NCI, DCP-sponsored nonclinical toxicology studies with Polyphenon E include: 28-day toxicity and 56-day mutagenicity in BigBlue transgenic mice; 13-week toxicity in rats and dogs; six-month oral carcinogenicity in p53 +/– mice; six-month toxicity in Sprague Dawley rats, nine-month toxicity in Beagle dogs, and teratology and reproductive toxicity in rats and rabbits. NCI, DCP-sponsored investigations with EGCG include 28-day and 13-week toxicity in rats and dogs. NCI, DCP has also sponsored mutagenicity and genetic toxicology studies with EGCG and Polyphenon E. (See NCI, DCP Investigator’s Brochure for more information.)

The NCI, DCP-sponsored nine-month oral toxicity study of Polyphenon E administered to fasted male and female Beagle dogs was terminated prematurely because of excessive loss of animals due to mortality and moribund sacrifice in all treatment groups (200, 500 and 1000/800 mg Polyphenon E/kg-bw/day). Gross necropsy revealed test-article related lesions in the gastrointestinal tract, liver, kidney, reproductive organs and hematopoietic tissues of treated male and female dogs. An investigation to determine the cause of the toxicity is ongoing; administration of the agent to fasted dogs may have been the cause of increased toxicity seen in the nine-month *vs*. the 13-week NCI, DCP-sponsored dog study. However, in a follow-up study in fed *vs.* fasted dogs, using several Polyphenon E formulations, no deaths occurred; numerous biochemical endpoints are currently being evaluated.

The safety of tea and tea compounds is supported by centuries of consumption by the human population. However, in recent years, oral use of green tea extracts (GTEs) has been associated with several instances of hepatotoxicity. In 2003, the sale of Exolise (an ethanolic GTE sold as a weight reduction aid) was suspended in Spain in after reports of hepatotoxicity (four cases in Spain and nine in France) associated with its use . Although hepatotoxicity in most cases resolved within four months of stopping GTE, there have been cases of positive rechallenge and liver failure requiring liver transplantation. A written safety report submitted to NCI, DCP-sponsored IND 58,367 in December 2005 described a case of acute liver failure in a woman consuming Green Lite®capsules distributed by Origin Biomedicinals, Inc., Halifax, Canada. Green Lite® capsules contain Polyphenon 70A, a GTE manufactured by Mitsui Norin, and similar in composition to Polyphenon E. This incident was subsequently reported as a published case report by members of Queen Elizabeth II Health Sciences Center, Dalhousie University (Halifax, Nova Scotia) . Since no other cause could be identified, the treating physicians concluded that the cause of fulminant liver failure experienced by this subject was most likely related to the consumption of over-the-counter GTE supplements for weight loss. Time to onset of hepatotoxicity following ingestion of GTEs ranged from several days to several months. Increased oral bioavailability occurs when GTEs are administered on an empty stomach after an overnight fast. Increased toxicity, including hepatotoxicity, is observed when Polyphenon E or EGCG is administered to fasted dogs. Therefore, the FDA Division of Drug Oncology Products has recommended that Polyphenon E be taken with food by subjects participating in clinical studies. In addition, subjects should have liver function tests performed at baseline and repeated every four weeks while on treatment. Following any elevation in ALT, study drug should be withheld (grade 1) or discontinued (grade ≥2), and liver function monitored until recovery to normal. Interferon-beta (Avonex®, Rebif®, Betaseron®) also cause rare cases of liver injury. Whether this risk could be increased by concurrent use of Polyphenon E is unclear but the monthly monitoring of liver tests should minimize it.

One case of grade 1 rectal bleeding occurred in a 56 year old participating in an investigator initiated study of Polyphenon E on endogenous hormones. The subjects reported a small amount of blood (about 5 drops) in the stool for 3-4 days that started 2 days after starting study drug. The blood was bright red so it was thought it came from a fissure or hemorrhoid but because of the close relationship with starting the study agent the AE was classified as possibly related. The bleeding resolved spontaneously and did not affect the hemoglobin or hematocrit.

A second case of rectal bleeding occurred in a 57-year-old female subject enrolled in the NCI, DCP-sponsored study entitled *A Phase Ib Randomized, Double-blinded, Placebo-controlled, Dose Escalation Study of Polyphenon E in Women with a History of Hormone Receptor-negative Breast Cancer* conducted by the University of Texas MD Anderson Cancer Center Phase I/II Chemoprevention Trial Consortium. The trial is investigating 400 mg, 600 mg and 800 mg EGCG bid provided by Polyphenon E *vs.* placebo. Approximately 18 days after starting the study medication the subject required hospital admission for rectaol bleeding that had begun two days earlier. The patient experienced an episode of bright red rectal bleeding associated with nausea, vomiting and abdominal pain. The bleeding continued for approximately nine more episodes. The subject was evaluated in the emergency room and admitted to the ICU. She was treated with blood transfusion of two units of blood. A colonoscopy revealed moderate to severe diverticulosis throughout the colon with no active bleeding. An esophagogastroduodenoscopy was negative for bleeding but revealed erosive gastritis in the gastric antrum and distal body.. Gastric biopsy revealed reactive gastropathy but was negative for *H. pylori.* The event resolved a day after admission and the subject was discharged home. The subject was 20nblended in response to the adverse event and was found to be taking study drug at 400 mg EGCG bid. The adverse event was thought to be possibly related to study medication.

**Availability**

Polyphenon E Capsules, 200 mg EGCG, is an investigational agent supplied by agreement through Polyphenon E International, Inc. (New York, NY).and NCI, DCP. It will be packaged and distributed by:

Fisher BioServices, Division of Cancer Prevention (DCP) Repository

20301 Century Blvd.

Building 6, Suite 800

Germantown, MD 20874

Phone (240) 686-4719

**Agent Distribution**

Agents will only be released after documentation of IRB approval of the protocol and informed consent is provided, and the collection of all required regulatory documents is complete. Collection of regulatory documents will be coordinated by the regulatory contractor, CCS Associates. When all required documents have been obtained, CCS Associates will notify NCI, DCP and Fisher BioServices, DCP Repository, that drug may be released for the study; the Principal Investigator and the individual at the study institution specified as responsible for receiving drug shipments will be copied on this correspondence. Fisher BioServices, DCP Repository, will then contact the specified pharmacy contact and provide him/her with instructions for ordering study agent. Shipments are generally made within five days of receiving a request.

**Agent Accountability**

The Principal Investigator, or a responsible party designated by the Principal Investigator, must maintain a careful record of the inventory and disposition of all agents received for the study. The investigator will be required to maintain adequate records of receipt, dispensing and final disposition of study drug. Included on receipt record (*e.g.,* packing slip) will be from and to whom study drug was shipped, date, quantity, and batch or lot number. Quantities and dates study agent was dispensed to and returned by each participant will be noted on a dispensing record. At completion of the investigation, unused drug will be destroyed. A record documenting the destruction of unused drug will include quantity, date, batch or code.

**Packaging and Labels**

Polyphenon E, 200 mg EGCG capsules, and matching placebo capsules are provided by Fisher BioServices, DCP Repository packaged with 100 capsules per bottle in 150 cc white HDPE bottles. Bottles will be labeled with blinded labels including agent name, dosing and storage instructions, required warnings for restricted, investigational use, and a space for recording the subject ID number.

**Storage**

Polyphenon E Capsules will be stored in a secure location at room temperature [between 59*°*F and 86*°*F (15-30*°*C)].

**Registration/Randomization**

**Pilot study**

Registration procedures will be the same as detailed below. There will be no randomization.

**Phase II study**

Each subject screened will be assigned a registration number upon signature of informed consent. The registration number will be assigned by the investigator or the research coordinator. The registration numbers will be consecutive numbers. The investigator or research coordinator will enter the subject’s name, birth date, contact information and clinic number in the database and the database will assign this record a serial number that will be the registration number. This database will be stored in a secure network drive and will be encrypted and password protected. The registration number will be the primary key for a second database containing the rest of the collected data. The registration number will be used as the identifier for all the subjects case report forms (CRF).

Upon randomization each subject will be assigned an individual randomization ID number. The allocation will be one to one, based on random permutations of a sequence with a block size of six. The randomization sequence will be stratified by disease type and treatment (GA or no treatment). A statistician in the LSU/Tulane/Medical Center of New Orleans General Clinical Research Center will generate the allocation sequence. The research pharmacist will allocate the subjects according to the sequence recording, the subject’s registration number, birth date and initials as identifiers in the pharmacists randomization log. The randomization number will be used also be included in all case report forms.

**Blinding and Unblinding Methods for Phase II Study**

Identical containers and instructions will be used to mask the subjects to treatment assignment. The evaluation of outcomes will be done without knowledge of treatment assignment or adverse events by having separate treating and examining physicians and research assistants. The examining physician will perform the baseline examination as well as the follow-up neurological exams. The examining research assistant will assist with these procedures and perform the MS functional composite. The treating physician will review the monitoring labs and perform the assessments for adverse events and decide when subjects should discontinue therapy according to the protocol. The treating research assistant assist will assist with the evaluation of adverse events. Subjects will be instructed to discuss adverse events only with the treating physician and research assistant and not with the examining physician or research assistant. Dr. Lovera will serve as the treating physician and Dr. Gutierrez (see attached letter) as the examining physician. The MRI data will be coded with a unique identifier different from the main study id code. The examining research assistant will strip the scan headers from the identifying information and replace them with this MRI code so that MRI outcome measurements can be performed without access to the clinical data. The processing of the data will occur after every five scans are acquired.

The DSMB can authorize to break the blind if there are safety concerns at any point. The statistician in the DSMB will have access to the allocation sequence and will be able to generate 23nblended analyses at the pre-specified safety monitoring time points. The DSMB chair can also request that allocation be revealed to him when reviewing AE reports.

Once recruitment is completed the database will be carefully reviewed for accuracy. After the database is clean it will be locked and no further changes will be allowed. The statistician will perform the analyses with treatment allocation coded as “A” and “B”; once all analyses are completed, the “A” and “B” code will be revealed.

**Agent Destruction/Disposal**

At the completion of the investigation, remaining drug will be destroyed on site per institutional SOPs.

# CLINICAL EVALUATIONS AND PROCEDURES

**Schedule of Events**

**Pilot study**

| **Intervention** |  |  | **Treatment Polyphenon E, 400 mg EGCG twice a day or placebo** | | | | | | | |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Visit number** | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|  | **Screening** | Day | | Month | | | | | | | |
| **Procedure** | **/Baseline Tests** |  | |  | | | | | | | |
|  | (Day -14 to 0) | 0 | 0-7 | 1 | 2 | 3 | 4 | 5 | 6 | 6 | 7 |
| Consent/registration | x |  |  |  |  |  |  |  |  |  |  |
| Chart review | x |  |  |  |  |  |  |  |  |  |  |
| Medical history | x |  |  |  |  |  |  |  |  |  |  |
| Vital signs | x |  |  |  |  | x |  |  | x |  |  |
| Weight/Height | x |  |  |  |  | x |  |  | x |  |  |
| Physical exam | x |  |  |  |  | x |  |  | x |  |  |
| EDSS | x |  |  |  |  |  |  |  | x |  |  |
| MSFC |  |  | x |  |  |  |  |  | x |  |  |
| MFIS |  |  | x |  |  |  |  |  | x |  |  |
| SF 36 |  |  | x |  |  |  |  |  | x |  |  |
| PDQ |  |  | x |  |  |  |  |  | x |  |  |
| MSNQ |  |  | x |  |  |  |  |  | x |  |  |
| BDI-II |  |  | x |  |  |  |  |  | x |  |  |
| Cognitive testing |  |  | x |  |  |  |  |  | x |  |  |
| Compliance check |  |  |  |  |  | x |  |  | x |  |  |
| Drug dispensing |  |  | x |  |  | x |  |  |  |  |  |
| Compliance check |  |  |  |  |  | x |  |  | x |  |  |
| Adverse Event Assessment |  |  |  | x | x | x | x | x | x |  | x |
| Continued next page  | | | | | | | | | | | |
| **Table 1** Schedule of events Phase II study |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |
| Concomitant Medications/Supplements/Tea | x |  |  | x | x | x | x | x | x |  |  |
| Blood draw | x |  |  | x | x | x | x | x | x |  |  |
| Peak and trough serum EGCG levels |  |  |  | x |  |  |  |  |  |  |  |
| Comprehensive metabolic panel1 | x |  |  |  | x | x |  |  | x |  |  |
| Liver panel2 | x |  |  | x | x | x | x | x | x |  |  |
| CBC with platelets | x |  |  | x | x | x | x | x | x |  |  |
| Pregnancy test | x |  |  |  |  |  |  |  |  |  |  |
| MRI |  | x |  |  |  |  |  |  |  | x |  |

**Table 1** Schedule of events Phase II study

Comprehensive metabolic panel includes: glucose, calcium, albumin, total protein, sodium, potassium, CO2, chloride.

Liver panel includes: AST, ALT, ALP, albumin, total bilirubin, direct bilirubin, total protein.

Performed by telephone contact.

**Phase II study**

| **Intervention** | Treatment Polyphenon E, 400 mg EGCG, twice a day or placebo | | | | | | | | | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Visit number** | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
| **Procedure** | **Screening** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | **/Baseline Tests** | Day | | Month | | | | | | | | | | | | | | |
|  | (Day -14 to 0) | 0 | 0-7 | 1 | 2 | 3 | 4 | 5 | 6 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 12 | 13 |
| Consent/registration | x |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Chart review | x |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Medical history | x |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Vital signs | x |  |  |  |  | x |  |  | x |  |  |  | x |  |  | x |  |  |
| Weight/Height | x |  |  |  |  | x |  |  | x |  |  |  | x |  |  | x |  |  |
| Physical exam | x |  |  |  |  | x |  |  | x |  |  |  | x |  |  | x |  |  |
| EDSS | x |  |  |  |  |  |  |  | x |  |  |  |  |  |  | x |  |  |
| MSFC |  |  | x |  |  |  |  |  | x |  |  |  |  |  |  | x |  |  |
| MFIS |  |  | x |  |  |  |  |  |  |  |  |  |  |  |  | x |  |  |
| SF 36 |  |  | x |  |  |  |  |  |  |  |  |  |  |  |  | x |  |  |
| PDQ |  |  | x |  |  |  |  |  |  |  |  |  |  |  |  | x |  |  |
| MSNQ |  |  | x |  |  |  |  |  |  |  |  |  |  |  |  | x |  |  |
| BDI-II |  |  | x |  |  |  |  |  |  |  |  |  |  |  |  | x |  |  |
| Cognitive testing |  |  | x |  |  |  |  |  |  |  |  |  |  |  |  | x |  |  |
| Randomization |  | x |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Drug dispensing |  |  | x |  |  | x |  |  | x |  |  |  | x |  |  | x |  |  |
| Compliance check |  |  |  |  |  | x |  |  | x |  |  |  | x |  |  | x |  |  |
| Adverse Event Assessment |  |  |  | x | x | x | x | x | x |  | x | x | x | x | x | x |  | x |
| Concomitant Medications/Supplements/Tea | x |  |  | x | x | x | x | x | x |  | x | x | x | x | x | x |  |  |
| Blood draw | x |  |  | x | x | x | x | x | x |  | x | x | x | x | x | x |  |  |
| Peak and trough serum EGCG levels |  |  |  | x |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Comprehensive metabolic panel1 | x |  |  |  | x | x |  |  | x |  |  |  |  |  |  | x |  |  |
| Liver panel2 | x |  |  | x | x | x | x | x |  |  | x |  | x | x | x | x |  |  |
| CBC with platelets | x |  |  |  | x | x | x | x | x |  | x |  | x | x | x | x |  |  |
| Smell test |  | x |  |  |  | x |  |  | x |  |  |  | x |  |  | x |  |  |
| Pregnancy test | x |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| MRI |  | x |  |  |  |  |  |  |  | x |  |  |  |  |  |  | x |  |

**Table 2** Schedule of events Phase II study

Comprehensive metabolic panel includes: glucose, calcium, albumin, total protein, sodium, potassium, CO2, chloride.

Liver panel includes: AST, ALT, ALP, albumin, total bilirubin, direct bilirubin, total protein.

Performed by telephone contact.

**Pilot study evaluations and visits**

**Baseline Testing/Pre-study Evaluation**

***Screening /Baseline Testing (visit 0):***

Obtain informed consent, assign a registration number and collect demographic information, review medical records, and measure vital signs, height and weight. The examining physician will perform a physical exam, obtain relevant medical history, and administer the EDSS. We will record recent tea consumption and all medications and supplements the subject is taking. Blood will be drawn (approx. 60 mL) for a comprehensive metabolic panel, liver panel, and CBC with platelets. A urine pregnancy test will be given to women of child-bearing potential. This visit will last approx. 1 hour.

Prestudy assessments, including labs, must be performed within 14 days of randomization/initial drug dispensing.

***Baseline Imaging (visit 1, day 0)***

If results from the blood tests and other screening visit procedures indicate that the subject is eligible for the study, we will inform him/her and schedule the baseline imaging visit. Subjects will undergo an MRI with gadolinium. This visit will last approximately two hours.

***Baseline cognitive and quality of life evaluation. (visit 2, day 0-7)***

If the subject is able to complete the scan, we will dispense the study drug. Subjects will be provided with enough study medication for the first three months of intervention (4 bottles containing 100 capsules per bottle). The subjects will be instructed to take two capsules each morning and evening with a meal, and educated on the signs and symptoms of liver toxicity.

The subjects will complete the SF 36, the PDQ, the MFIS and the BDI-II questionnaires as well as the cognitive tests, and complete the MSFC. The MSNQ will be given to the subject to have a family member complete it and return it the next visit. We will record any recent tea consumption, and any changes to medications and supplements that the subject is taking. Any pretreatment adverse events will be recorded.

This visit will last approximately two hours.

**Evaluations During Study Intervention**

***Plasma levels of EGCG (Month 1, visit 3):***

After one month on the full dose of the drug the subjects will have peak and trough levels of EGCG in the plasma measured. For this visit the subjects will arrive at the CTRC before their breakfast and morning dose of study drug. The subjects will receive a standardized breakfast and will be supervised while taking the capsules. We will collect blood three and eight hours after the dose in tubes containing EDTA. Plasma will be isolated by centrifugation and combined with one-tenth the volume of a preservative solution (20% ascorbic acid and 0.05% Na2EDTA in 0.4 M phosphate buffer, pH 7.2) to prevent the degradation of EGCG. The plasma with preservative will be divided in aliquots and frozen at -80ºC until analyzed by HPLC.

Blood will be sent for liver function tests. The subjects will be provided with a calendar to record daily intake of the study drug and record adverse events. We will review the calendar and record the number of doses taken and adverse events reported. The research assistant will review the concomitant medication list and record any medication changes and green tea intake.

***Monitoring visits (visits4-7) (monthly up to end of treatment, months 2-5)***

The total time for the visits will be 30-90 min. Activities will vary depending on month number.

**Blood draw and adverse event assessment (monthly)**

Blood will be drawn for liver function tests. The subjects will be provided with a calendar to record daily intake of the study drug and record adverse events. We will review the calendar and record the number of doses taken and adverse events reported. The research assistant will review the concomitant medication list and record any medication changes and green tea intake. The labs that will be done at each visit are detailed in section . All labs will be reviewed by the treating physician within three days of being processed by the lab.

This portion of the visit will last 30 min.

**Physical exam, compliance monitoring and dispensing (every three months):**

A general physical exam will be done by the treating physician. Vital signs and weight will be measured. We will review compliance by pill counts. Additional drug for three months will be dispensedThis portion of the visit will last 30 min

**Adverse events:**

Adverse events will be recorded using the verbatim term as well as categories and grading by the Cancer Therapy Evaluation Program (CTEP) Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0. The treating physician will assign the categories and grading.

The treating physician will perform the evaluations of adverse events. When the research assistant records any adverse event during the monthly monitoring visit he or she will notify the treating physician. Those subjects who are experiencing an adverse event potentially related to therapy with a severity grade of three or higher will be evaluated in an unscheduled visit within 24 hours. Subjects experiencing adverse events of lesser grades will be evaluated in unscheduled visits at the discretion of the treating physician. The treating physician will arrange for coverage in case of vacation or leave.

**MS relapse evaluations**

Relapses are defined as the occurrence of a new neurological symptom or worsening of an old one, with an objective change of at least one point in the functional system scale score from the EDSS, lasting at least 24 h, without fever, and which followed a period of clinical stability or of improvement of at least 30 days.

A subject who suspects a relapse will be evaluated within 72 hours of onset. The treating physician will perform an EDSS and the research assistant will perform the MSFC. The treating physician will evaluate the subject and determine if the relapse is resulting in a significant impairment in the subject’s quality of life. If this is the case then the subject will be treated with methylprednisolone (1 gm/day for three days). Subjects who relapse will be evaluated one month after the onset of the relapse to document the extent of improvement.

For each relapse the following information will be recorded: date of onset of the relapse, date of the exam, and treatment. EDSS and MSFC for the relapse visit and the 1 month postrelapse visit will also be recorded.

**Evaluations at Completion of Study Intervention**

***End of treatment MRI. (Month 6, visit 8)***

The end-of-treatment MRI with gadolinium will be performed. The visit will last 2 hours. This visit can occur within 15 days of the specified time point to allow scheduling flexibility.

***End of treatment clinical and cognitive evaluation (Month 6, Visit 9):***

The research assistant will record any adverse events, review the medication/supplement list, and record any medication changes or recent tea consumption.

The treating physician will do a physical exam and will administer the EDSS. The MSFC will be administered by the research assistant. Vital signs and weight will be measured.

The subject will complete the cognitive battery and the MFIS, SF 36, PDQ and the BDI-II questionnaires. The subject will report if they think the treatment has been effective. The MSNQ questionnaire will be given to the subject to have a family member or caregiver complete and return by mail. Remaining study drug will be collected and counted for a final measure of compliance.

**Post-intervention Follow-up Period Post-treatment Day 30 (Visit 10):**

Patients will be followed as indicated by usual care practices after the study. One month after ending treatment, subjects will be contacted by telephone to determine whether any adverse symptoms have developed or worsened since stopping drug. If adverse events have occurred, information on concomitant medications and supplements will also be collected to aid in the assessment of study drug-relatedness. No other post-study endpoints will be collected.

**Phase II Study**

**Phase II study evaluations and visits**

**Baseline Testing/Pre-study Evaluation**

***Screening /Baseline Testing (visit 0):***

Obtain informed consent, assign a registration number and collect demographic information, review medical records, and measure vital signs, height and weight. The examining physician will perform a physical exam, obtain relevant medical history, and administer the EDSS. We will record recent tea consumption and all medications and supplements the subject is taking. Blood will be drawn (approx. 60 mL) for a comprehensive metabolic panel, liver panel, and CBC with platelets. A urine pregnancy test will be given to women of child-bearing potential. This visit will last approx. 1 hour.

Prestudy assessments, including labs, must be performed within 14 days of randomization/initial drug dispensing.

***Baseline Imaging/Randomization (visit 1, day 0)***

If results from the blood tests and other screening visit procedures indicate that the subject is eligible for the study, we will inform him/her and schedule the baseline imaging visit. Subjects will undergo an MRI with gadolinium at Doctors Imaging Service. This visit will last approximately two hours.

***Baseline cognitive and quality of life evaluation. (visit 2, day 0-7)***

If the subject is able to complete the scan, he/she will be randomized and we will dispense the study drug. Subjects will be provided with enough study medication for the first three months of intervention (4 bottles containing 100 capsules per bottle). The subjects will be instructed to take two capsules each morning and evening with a meal, and educated on the signs and symptoms of liver toxicity.

The subjects will complete the SF 36, the PDQ, the MFIS and the BDI-II questionnaires as well as the cognitive tests, a smell test, and complete the MSFC. The MSNQ will be given to the subject to have a family member complete it and return it the next visit. We will record any recent tea consumption, and any changes to medications and supplements that the subject is taking. Any pretreatment adverse events will be recorded.

This visit will last approximately two hours.

**Evaluations During Study Intervention**

***Plasma levels of EGCG (Month 1, visit 3):***

After one month on the full dose of the drug the subjects will have peak and trough levels of EGCG in the plasma measured. For this visit the subjects will arrive at the CTRC before their breakfast and morning dose of study drug. The subjects will receive a standardized breakfast and will be supervised while taking the capsules. We will collect blood three and eight hours after the dose in tubes containing EDTA. Plasma will be isolated by centrifugation and combined with one-tenth the volume of a preservative solution (20% ascorbic acid and 0.05% Na2EDTA in 0.4 M phosphate buffer, pH 7.2) to prevent the degradation of EGCG. The plasma with preservative will be divided in aliquots and frozen at -80ºC until analyzed by HPLC.

Blood will be sent for liver function tests. The subjects will be provided with a calendar to record daily intake of the study drug and record adverse events. The treating research assistant will review the calendar and record the number of doses taken and adverse events reported. The research assistant will review the concomitant medication list and record any medication changes and green tea intake.

***Monitoring visits (visits4-14) (monthly up to end of treatment, months 2-11)***

The total time for the visits will be 30-90 min. Activities will vary depending on month number.

***Smell test (every three months)***

**Olfactory senses will be tested to ensure that there is no decreased sensation or loss of function. This portion of the visit will last 5 minutes.**The smell test will be provided by Sensonics Inc. and is called the Modified Brief Identification Smell Test. Tasks for subjects will include odor differentiation by strength of odor and whether an odor is pleasant or aversive.

***Blood draw and adverse event assessment (monthly)***

Blood will be drawn for liver function tests. The subjects will be provided with a calendar to record daily intake of the study drug and record adverse events. The treating research assistant will review the calendar and record the number of doses taken and adverse events reported. The research assistant will review the concomitant medication list and record any medication changes and green tea intake. The labs that will be done at each visit are detailed in section . All labs will be reviewed by the treating physician within three days of being processed by the lab.

This portion of the visit will last 30 min.

#### Physical exam, compliance monitoring and dispensing (every three months):

A general physical exam will be done by the treating physician. Vital signs and weight will be measured. We will review compliance by pill counts. Additional drug for three months will be dispensed. This portion of the visit will last 30 min

***EDSS and MSFC evaluation (every 6 months)***

The examining physician will perform the EDSS and the research assistant will perform the MSFC.

***End of treatment MRI. (Month 12, visit 15)***

The end-of-treatment MRI with gadolinium will be performed at LSU-HSC. The visit will last 2 hours. This visit can occur within 15 days of the specified time point to allow scheduling flexibility.

#### End of treatment clinical and cognitive evaluation

The treating physician will do a physical exam and the treating physician will administer the EDSS. The MSFC will be administered by the research assistant. Vital signs and weight will be measured.

The subject will complete the cognitive battery and the MFIS, SF 36, PDQ and the BDI-II questionnaires. The subject will report if they think the treatment has been effective. The MSNQ questionnaire will be given to the subject to have a family member or caregiver complete and return by mail. Remaining study drug will be collected and counted for a final measure of compliance. Finally, the physicians and the research assistant will complete a form indicating which treatment they think the subject was assigned to and why.

### Post-intervention Follow-up Period Post-treatment Day 30 (Visit 17):

Patients will be followed as indicated by usual care practices after the study. One month after ending treatment, subjects will be contacted by telephone to determine whether any adverse symptoms have developed or worsened since stopping drug. If adverse events have occurred, information on concomitant medications and supplements will also be collected to aid in the assessment of study drug-relatedness. No other post-study endpoints will be collected.

**Adverse events:**

Adverse events will be recorded using the verbatim term as well as categories and grading by the Cancer Therapy Evaluation Program (CTEP) Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0. The treating physician will assign the categories and grading.

The treating physician will perform the evaluations of adverse events. When the research assistant records any adverse event during the monthly monitoring visit he or she will notify the treating physician. Those subjects who are experiencing an adverse event potentially related to therapy with a severity grade of three or higher will be evaluated in an unscheduled visit within 24 hours. Subjects experiencing adverse events of lesser grades will be evaluated in unscheduled visits at the discretion of the treating physician. The treating physician will arrange for coverage in case of vacation or leave.

**MS relapse evaluations**

Relapses are defined as the occurrence of a new neurological symptom or worsening of an old one, with an objective change of at least one point in the functional system scale score from the EDSS, lasting at least 24 h, without fever, and which followed a period of clinical stability or of improvement of at least 30 days.

A subject who suspects a relapse will be evaluated within 72 hours of onset. The examining physician will perform an EDSS and the examining research assistant will perform the MSFC. The treating physician will evaluate the subject and determine if the relapse is resulting in a significant impairment in the subject’s quality of life. If this is the case then the subject will be treated with methylprednisolone (1 gm/day for three days). Subjects who relapse will be evaluated one month after the onset of the relapse to document the extent of improvement.

For each relapse the following information will be recorded: date of onset of the relapse, date of the exam, and treatment. EDSS and MSFC for the relapse visit and the 1 month postrelapse visit will also be recorded.

**Methods for Clinical Procedures**

**Laboratory Tests**

Blood counts and chemistries will be performed by University Hospital through the Clinical Transalational Research Center (CTRC) according to standard clinical protocols.

**MRI**

***Conventional MRI***

MPRAGE data will be collected with sub-millimeter in plane resolution and 2 mm or less effective slice thickness. T2 and proton density (PD) will be collected using 3 mm slice thickness. Whole-brain coverage will be provided with typical field of view (FOV) of 180 mm x 240 mm x 144 mm or better.

***Active lesion determination***

The number of combined unique lesions will be determined by manual counting avoiding double counting when an enhancing lesion corresponds to a new or enlarging lesion. Lesions will be manually segmented and classified as active or chronic lesions and masks will then be created for each lesion.

***Proton Spectroscopy***

Two10 mm thick axial planes of water suppressed 1H magnetic resonance spectroscopic imaging will be collected, one at the level of the centrum semiovale and one at the level of the thalamus. A short echo time point-resolved spectroscopy technique will be used for inner volume selection that will be maximized for parenchymal detection while minimizing extracranial excitation. Outer volume saturation pulses will be used to provide addition suppression of extracranial lipid. The effective echo time will be 30 msec or better, sufficient to capture all singlet and most multiplet resonances. A second acquisition for each of the slices will be performed without water suppression to allow for the determination of the absolute metabolite concentrations. In follow up scans, the slices will be positioned by aligning the high resolution anatomical image obtained in the initial scan with the one obtained in the follow-up scan using the tools available in the scanner for this purpose. We are standardizing a protocol to use the proton density data to determine the water content; if we successfully implement this approach then we will be able to obtain four axial slices.

***Quantitative T1***

We will follow the general approach described by Clare and Jezzard for fast T1 measurement at high-field. A non-selective adiabatic inversion pulse will be used to invert magnetization followed by a slice selective sampling pulse and EPI readout. Twenty-four 3 mm slices will be selected with a 45º sampling optimized sinc- pulse. Twenty-four inversion times (TI, linear array ranging from 120 msec to 5640 msec) will be sampled for each slice with an effective TR of 6s. The image matrix will be 1282 over a (192 mm)2 FOV. The nominal resolution will be (1.5 mm)2 x 3 mm and total coverage will be 76 mm, sufficient to sample the majority of supratentorial white matter in most subjects. The peak and mean T1 values will be determined from the analysis of each subject’s whole brain histogram at each time point.

*Contrast enhanced MRI*

A dose of 0.1 mmol per KG will be administered IV. Five minutes after IV contrast administration we will obtain axial T1 weighted images using 3 mm slice thickness. Whole-brain coverage will be provided with typical field of view (FOV) of 180 mm x 240 mm x 144 mm or better.

***Atrophy***

Percentage brain volume change will be estimated from the anatomical scans using Structural Image Evaluation, using Normalization, of Atrophy (SIENA) http://www.fmrib.ox.ac.uk/fsl/siena. This is an accepted fully automated method that has an accuracy of 0.2%.

***Quality assurance***

All MRI scans will be performed on the 3T Siemens Trio MRI scanner at Doctors Imaging Services (Metairie, New Orleans), which is used exclusively for research. A quality assurance scan of a known phantom using the protocol MRS/I techniques will be performed every six months. The scanner is new and it is not anticipated to change over the duration of the study or require major maintenance, but if it does we will also perform a quality assurance scan of a known phantom before scanning any further subjects. The software updates to the scanner will remain restricted. A backup of the initial software configuration will be maintained for use throughout the study in case of a software update.

***Experimental sequences***

Some of the sequences that will be used in this study are investigational and have not been fully validated by the FDA. The use of these sequences does not represent a risk to the patient.

**Cognitive testing**

The cognitive testing battery will consist of four tests, The Stroop test, the PASAT, the COWAT and the CVLT. The cognitive domains evaluated by these tests are frequently impaired in MS. For all four tests that form the battery, we will use different but comparable versions for the two testing sessions. This will minimize learning effects.

We will also administer the WRAT test. We will use this test to measure pre-morbid intelligence.

***The Stroop Color and Word Test***

The Stoop Color and Word Test is a measure of concentration and attention. Patients are shown a series of cards with a list of words printed in different color ink. The task is to name the color of each word. The time taken to complete the condition 3 of the Stroop test will be the measurement used for the primary outcome. Results on the first two conditions will be analyzed separately.

***PASAT***

This is a measure of working memory and sustained attention frequently used in MS treatment outcome studies. The examinee is presented with a series of numbers at 2-second intervals on an audiotape and responds by always adding the last two numbers on the tape before the next number is presented. The total number of correct responses will be the measurement used for the primary outcome.

***Controlled Oral Word Association Test (COWAT)***

This is a letter fluency test. Participants are asked to generate as many words as possible beginning with a particular letter of the alphabet during one minute. Alternate versions using three letters are used for each examination. The total number of words produced for the three letters will be the primary outcome.

***California Verbal Learning Test (CVLT-II)***

This test is a measure of verbal learning/memory. It is comprised of lists containing sixteen words each of which fit into one of four categories of “shopping list” items. Five trials are administered followed by presentation of a different list. Free and cued recall of the original list is then assessed. The long delay free recall will be the measurement used for the primary outcome measure. Other measurements that are part of the CVLT-II will also be analyzed but will not be part of the primary outcome.

***Wide Range Achievement Test (WRAT-R2)***

This test is a measure of reading ability. Reading ability in general verbal IQ is affected the least by pathological conditions and thus serves as a good measure of premorbid intelligence. The test consists of a series of words with varying degrees of vocabulary difficulty. The subject is asked to read them and the score of the test is the number of words correctly pronounced, this test will be used as a covariate in the analysis but not as an outcome measure.

**Questionnaires**

***Beck Depression Inventory II (BDI)***

The BDI is a validated self-report measure of depression that follows the DSM-IV criteria for depression. In addition, we will determine whether changes in depression may explain any changes in cognitive performance.

***Perceived Deficits Questionnaire (PDQ)***

The PDQ is part of the MSQLI. The PDQ consists of twenty items addressing cognitive difficulties over four subscales. Subjects rate their responses over a five-point scale ranging from one (never) to five (almost always). The items correspond to four subscales. From our pilot data the improvements on the Retrospective Memory Subscale from this questionnaire correlate with improvements on the Stroop test.

***Multiple Sclerosis Neuropsychological Questionnaire (MSNQ)***

This is a questionnaire where the family member or caregiver reports on the subject’s cognitive deficits. This MSNQ has been validated in people with MS and correlates significantly with the performance on a cognitive test battery.

***Modified Fatigue Impact Scale (MFIS)***

The MFIS is a multidimensional fatigue scale that has been used in several clinical trials and is part of the MSQLI. We will use the results to also assess whether changes in fatigue may explain any changes in cognitive performance.

***The Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36)***

The SF-36 is a measure of health status designed for use in clinical practice, research, health policy evaluations, and general population surveys. It includes eight scales that assess the following general health concepts: physical functioning, role limitations due to physical health problems, bodily pain, general health, perceptions, vitality, social functioning, role limitations due to emotional problems, emotional, and mental health.

# RISKS AND PROVISIONS TO MINIMIZE THEM

Polyphenon E is well tolerated and frequent side effects with this agent are mild. It is unclear whether Polyphenon E has the potential for rare liver toxicity as has occurred with other green tea extracts. This serious complication has not occurred in the clinical trials done with Polyphenon E up until now. However, careful monitoring for this potential complication is in place in this study following the FDA recommendations and the study drug is taken by subjects with meals as this may have been a factor in the cases of liver toxicity with green tea compounds used for weight loss. It is likely that if any liver complications were to occur they would be detected early. One case of self limited rectal bleeding has occurred in one of the studies and is thought to be possibly related to Polyphenon E. The subjects will be monitored with CBC’s. The subjects will be questioned about rectal bleeding at the monthly monitoring visits. Other side effects from Polyphenon E not seen up to date could occur. Polyphenon E is not teratogenic in animal studies. However, the risks to a fetus are unknown. We check women for pregnancy at the start of the study. We instruct subjects that they must use a reliable form of contraception for the whole duration of the study. There is careful monitoring in place in to detect any other unforeseen complications. An independent Data Safety and Monitoring Board will monitor the study to ensure further protection of the subjects.

Breach of confidentiality is a risk of participating in this study. To prevent disclosure of health information the study case report forms (CRF) are only coded with the registration and randomization ID’s. The consent forms and other CRF will be maintained in locked cabinets in a secure office. The database containing the study data will only contain registration and randomization ID’s as identifiers. The database will be kept in a secure network location and will be encrypted and password protected.

The key linking the subject’s identifiers and the registration and randomization numbers will be maintained in a separate database with the same security protections. The identifying key will be destroyed at the end of the study.

The study evaluations present some risk of mild discomforts. Blood draws can be painful; MRI exams are noisy and may bother claustrophobic subjects; and the cognitive testing and questionnaires may upset subjects. More serious possible complications from exam procedures are falls during gait testing, infection of the venipuncture sites and reactions to gadolinium. The study personal are well trained in performing the study procedures in a safe way and in enhancing subject comfort. The subject’s renal function is checked as part of the baseline evaluation to prevent the complications gadolinium can cause in subjects with renal failure.

Other allergic reactions to gadolinium are extremely rare. MRI magnets can cause complications in subjects with metal in their bodies. It is routine to administer a screening questionnaire regarding metal objects before doing the MRI scans.

# POTENTIAL BENEFITS

It is unclear whether Polyphenon E has any benefits for subjects with MS. If indeed Polyphenon E is neuroprotective the subjects that receive active treatment may benefit and the subjects and other MS patients will benefit from the development of a new treatment for MS.

# CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

**Pilot Study Endpoints**

**Primary Endpoint**

The primary safety endpoint will be the frequency and severity of AEs.

**Exploratory Endpoints**

Exploratory imaging efficacy endpoints will be a comparison between baseline and exit on: brain NAA level as measured by MR Spectroscopy, change in peak T1 times, and the number of combined unique lesions.

Exploratory imaging endpoints will be a comparison between the two groups in the: changes in NAA in normal appearing white matter, gray matter, active lesions and chronic lesions.

Exploratory clinically efficacy endpoints are the proportion of subjects experiencing worsening on the EDSS greater than one point sustained over six months, and the change in MSFC score between baseline and exit.

As an exploratory endpoint the serum levels of EGCG will be correlated with the changes in NAA and other imaging parameters. EGCG levels will also be correlated with the occurrence of adverse events.

**Phase II Study Endpoints**

**Primary Endpoint**

The primary efficacy endpoint will be a comparison between groups in the rate of change in brain NAA level as measured by MR Spectroscopy. The primary safety endpoint will be a comparison between arms in the frequency and severity of AEs.

**Secondary Endpoints**

Secondary imaging efficacy endpoints will be a comparison between the two groups in: the rate of brain atrophy, the rate of change in peak T1 times, and the number of combined unique lesions.

Secondary clinically efficacy endpoints are comparisons between the two groups in: the number of relapses, the proportion of subjects experiencing worsening on the EDSS greater than one point sustained over six months, and the change in MSFC score.

**Exploratory Endpoints**

Exploratory imaging endpoints will be a comparison between the two groups in the: changes in NAA in normal appearing white matter, gray matter, active lesions and chronic lesions.

As an exploratory endpoint the serum levels of EGCG will be correlated with the changes in NAA and other imaging parameters. EGCG levels will also be correlated with the occurrence of adverse events.

**Criteria for Discontinuation of Study Agent**

Subjects may discontinue study agent (but may still remain on study) for the following reasons:

Completed the protocol-prescribed intervention

Adverse event or serious adverse event

Inadequate agent supply

Noncompliance

Subject’s choice

Medical contraindication

At the discretion of the Investigator

If the subject is willing, he/she will continue to be followed for safety reasons and in order to collect endpoint data according to the schedule of events.

**Termination/Withdrawal**

Participants may go ‘off-study’ for the following reasons:

Not meeting eligibility criteria

The protocol intervention and any protocol-required follow-up period is completed

Adverse event or serious adverse event

Lost to follow-up

Noncompliance

Consent is withdrawn

At the discretion of the Investigator

Death

# SPECIMEN MANAGEMENT

**Laboratories**

Blood counts and chemistries will be performed by the University Hospital clinical lab through the Clinical Translational Research Center (CTRC) according to standard clinical protocols.

**Collection and Handling Procedures**

CMP/Liver set: Collect 4 ml blood in GREEN top, lithium heparin tube. Label with conventional hospital labels. Send to hospital lab. Log date, time, patient ID visit and sample ID number contained in hospital label.

CBC: 3.0 ml blood, LAVENDER top Vacutainer® containing EDTA. Label with conventional hospital labels. Send to hospital lab. Log date, time, patient ID visit and sample ID number contained in hospital label.

Plasma EGCG: two LAVENDER top Vacutainer containing tubes. Label with conventional hospital labels. Send to CTRC. Log date, time, time since last dose, patient ID, visit and sample ID number contained in hospital label. CTRC lab will isolate plasma by centrifugation the plasma will be combined with one-tenth the volume of a preservative solution (20% ascorbic acid and 0.05% Na2EDTA in 0.4 M PGX, pH 7.2), aliquoted and frozen at -80°C until analyzed by HPLC. Cryotubes will be labeled with study ID, sample, collection time (3h, 8h) and date.

# REPORTING ADVERSE EVENTS

DEFINITION: An adverse event (AE) is any untoward medical occurrence in a study participant. An AE does not necessarily have a causal relationship with the treatment or study participant. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with participation in a study, whether or not related to that participation. This includes all deaths that occur while a participant is on a study.

A list of adverse events possibly related to study agent that have occurred in completed NCI, DCP-sponsored Phase I investigations can be found in Section 6.2, Pharmaceutical Information; please also see the Investigator Brochure for additional information.

**Adverse Events**

**Reportable Adverse Events**

All adverse events that occur after the informed consent is signed (including during the screening process) and through the end of the follow-up period (or until a subject goes off study) must be recorded on the adverse event CRF (paper and/or electronic) whether or not related to study agent.

**Data Elements:**

AE reported date

AE Verbatim Term

CTCAE term (v3.0)

Event onset date and event ended date

Severity grade

Attribution to study agent (relatedness)

Whether or not the event was reported as a Serious Adverse Event (SAE)

Action taken with the study agent

Whether or not the subject dropped because of the event

Event status

Comments

**Classification and Severity Grading of AEs**

Identify the adverse event using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. The CTCAE provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTCAE can be found at http://ctep.cancer.gov.

AEs will be assessed according to the CTCAE grade associated with the AE term. AEs that do not have a corresponding CTCAE term will be assessed according to their impact on the participant’s ability to perform daily activities as follows:

|  |  |  |
| --- | --- | --- |
| **Grade** | **Severity** | **Description** |
| 1 | Mild | Barely noticeable, does not influence functioning  Causing no limitations of usual activities |
| 2 | Moderate | Makes participant uncomfortable, influences functioning  Causing some limitations of usual activities |
| 3 | Severe | Severe discomfort, treatment needed  Severe and undesirable, causing inability to carry out usual activities |
| 4 | Life-threatening | Immediate risk of death  Life-threatening or disabling |
| 5 | Fatal | Causes death of the participant |

**Assessment of relationship of AE to treatment**

The possibility that the adverse event is related to study drug will be classified as one of the following: not related, unlikely, possible, probable, definite.

**Follow-up of AEs**

All AEs, including lab abnormalities that in the opinion of the investigator are clinically significant, will be followed according to good medical practices and documented as such.

**Serious Adverse Events**

**Definition:**

ICH Guideline E2A and Fed. Reg. 62, Oct. 7, 1997 define serious adverse events as those events, occurring at any dose, which meet any of the following criteria:

Results in death

Is life-threatening (Note: the term life-threatening refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).

Requires inpatient hospitalization or prolongation of existing hospitalization

Results in persistent or significant disability/incapacity

Is a congenital abnormality/birth defect

Events that may not meet these criteria, but which the investigator finds very unusual and/or potentially serious, will also be reported in the same manner.

**Reporting Serious Adverse Events**

A serious adverse event, regardless of relatedness or expectedness, will be reported to CCSA by telephone within 24 hours of the PI/study team becoming aware of the event. A Serious Adverse Event (SAE) form will be completed by the investigator and faxed to CCSA within 48 hours. The investigator will keep a copy of this SAE form on file at the study site. SAEs will be reported by telephone and facsimile to:

Safety Department

CCS Associates, Inc.

2005 Landings Drive

Mountain View, CA 94043

Tel (650) 691-4400

Fax (650) 691-4410

The following information will be captured on the SAE form when reporting SAEs:

Date and time of the SAE

Date and time of the SAE report

Name of reporter

Call back phone number

Affiliation/Institution conducting the study

Protocol number

Title of protocol

Description of the SAE, including attribution to drug and expectedness

**IRB Notification by Investigator**

Reports of all SAEs (including follow-up information) will be submitted to the IRB per committee guidelines. Fatal and life-threatening local events will be reported within 48 hours, other serious adverse events within 5 working days. Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator’s binder.

**Follow-up of SAEs**

Site staff will send follow-up reports as requested and when additional information is available. Additional information will be entered on the SAE form in the appropriate format. Follow-up information will be sent to CCSA as soon as possible. Whenever possible, SAEs will be followed until resolved, especially for events related to the study agent.

# STUDY MONITORING

**Data Management**

CRFs will be completed by the study coordinator (SC). Edits check for valid values and ranges will be made real-time at entry. All records will be checked by hand for accuracy and completeness. The CRFs will be managed and housed in an area designated by the Principal Investigator. Computer files will be backed on regular intervals, long term storage of these files will be at an off-site location. Informed consent documents will be kept in files in an area designated by the Principal Investigator in a locked cabinet in a lock office.

The investigator will permit study-related monitoring, auditing, and inspection by the IRB, Polyphenon E International, Inc., and government regulatory bodies of all study related documents (*e.g*., source documents, regulatory documents, laboratory data, data collection instruments, etc.). The investigator will ensure the capability for inspection of all applicable study-related facilities (*e.g*., clinic, diagnostic laboratory, *etc*.).

**Case Report Forms**

Participant data will be collected using protocol-specific case CRFs. CRFs may require changes throughout the conduct of the clinical trial. The need for change may result from protocol amendments or other reasons.

**Source Documents**

Study observations and evaluations will be recorded in the medical records of subjects and then transcribed to study CRFs. Source documentation used to complete the CRFs include: chart notes (*e.g.,* physician notes, clinic notes, nursing notes, admission notes, *etc*.); verbal and written communication as documented in the research records; medical records; ancillary documents (such as x-rays, labs, scans, *etc*.); medication administration records; as well as computerized/electronic records and reports. All source documents will comply with the 21 CFR312.62 and FDA Guidance: E6 GCP Sections 1?6.

**Data and Safety Monitoring Plan**

**Pilot study:**

The study will be conducted in accordance with a Data and Safety Monitoring Plan (DSMP) appendix D.

The study will be monitored continuously and stopped if any case of liver failure (grade 3 or higher) or death due to liver disease thought to be possibly, probably or definitely related to study drug occurs in the active treatment arm.

The study will be monitored at two time points for the number of subjects in the treatment group with severe elevations (grade 3 or higher) in liver function enzymes (ALT, AST) or bilirubin; a risk of 1/10 would be acceptable for this side effect.

**Phase II study**

The study will be conducted in accordance with a Data and Safety Monitoring Plan (DSMP) appendix D.

The study will be monitored continuously and stopped if any case of liver failure (grade 3 or higher) or death due to liver disease thought to be possibly, probably or definitely related to study drug occurs in the active treatment arm.

The study will be monitored at six time points for the number of subjects in the treatment group with severe elevations (grade 3 or higher) in liver function enzymes (ALT, AST) or bilirubin; a risk of 1/10 would be acceptable for this side effect.

The study will be monitored for increase in disease activity (number of relapses, MRI activity) at the following three time points: after 50% of subjects complete three months in the study; when all subjects complete three months in the study; when all subjects complete one year in the study.

The study will be monitored for increased disability (EDSS, MSFC) when 100% of planned subjects have been enrolled, and when all subjects have completed one year of treatment.

**Record retention**

The principal investigator will retain study essential documents (including regulatory, laboratory data, case report forms, and source documents) for at least 2 years after the last approval of marketing applications in their county, or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement. CCSA (or Polyphenon E International, Inc.) will notify the investigator in writing when the documents can be destroyed.

**Confidentiality**

Information about the study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Additionally, subject will not be identified by name, only subject identification numbers, on specimens and questionnaires.

**Publication Plan**

No portion of the information provided by Polyphenon E International, Inc. for the purposes of performing the study will be published or passed on to any third party without the consent of Polyphenon E International, Inc. The principal investigator will have final responsibility for the decision to submit for publication.

# STATISTICAL CONSIDERATIONS

**Pilot study**

The primary objective of the pilot phase is obtaining preliminary safety data in subjects with MS. With 10 subjects the probability of stopping the study early at the predetermined interim analysis point or indicating that the next phase should not be conducted is 45% if the probability of serious complications (grade 3 or higher) is 30% or higher and would be less than 2% if the probability of serious complications is less than 10%.

**Phase II Study**

**Sample Size/Accrual Rate**

The minimum difference of interest is based on the two year where changes in NAA:Cr in subjects starting therapy with GA were compared with subjects refusing. The treated group experienced an increase from baseline of 0.21 units (11%) in their NAA:Cr ratios and the untreated group experienced a decline from baseline of 0.18 units (9%) in their NAA:Cr ratios resulting in a difference in the change on NAA:Cr ratios of 0.39 (20%). We will use absolute brain NAA levels in our study as opposed to the NAA:Cr ratio. Assuming that creatine remains constant during the study period, the percentage changes should still be valid.

Khan et al. did not explicitly report the standard deviation (SD) for the change in NAA in their paper. They used a non-parametric test so the SD cannot be determined from the p-value and sample size. We have used a conservative assumption that the correlation coefficient between baseline and exit is 0.5 and used the SD reported for the GA group at exit (0.26) to calculate the SD in the change of NAA over two years. We do not expect to see a difference between the two groups as large as the one seen in the study by Khan et al. as most of the subjects in both our groups will be on GA. We think that a difference of two thirds as large as the difference seen by Khan et al. would be clinically significant. This would be a 13% relative difference between the groups or corresponding 0.25 units in the NAA:Cr ratios. Smaller differences than this are not likely to be clinically significant since the effect of GA on disease progression is not large . Seventeen subjects per group are required to detect a relative difference between the two groups of 13% (0.25 units) with 80% power at an alpha level of 0.05 for a two-sided t-test assuming that the correlation coefficient between baseline and follow-up is 0.5. Assuming 10% non-compliance and 10% loss to follow up we would need 23 subjects per group.

**Randomization and Stratification**

The allocation will be one to one, based on random permutations of a sequence with a block size of six. The randomization sequence will be stratified by disease type and treatment (GA or no treatment). A statistician in the CTRC will generate the allocation sequence; a study coordinator will allocate the subjects according to the sequence.

**Analysis plan**

The analysis of all primary outcomes will be intent to treat and will include all available measures on all randomized subjects

***Primary Objectives:***

*Determine if treatment with Polyphenon E, 400 mg EGCG, twice a day for one year alters the rate of change of brain NAA levels in subjects with MS compared with those taking placebo.*

We will use a mixed model for the analysis of global NAA. A mixed model will permit using all available information for subjects that are lost to follow up. The model will include random effects for the intercept and the slope, nested within treatment, and fixed effects for treatment, time and treatment\*time. The main outcome will be the test for the treatment\*time interaction that indicates a difference in the slopes. The alpha level for the analysis will be 0.05 and the tests will be two sided. This will be the primary outcome of the study.

*Determine the safety of treatment with Polyphenon E at a dose containing 400 mg of EGCG twice a day over one year in subjects with MS.*

We will compare the differences in frequencies between the two groups in all adverse events and all serious adverse events with a severity of three or higher. We will tabulate all adverse events by category and compare the differences in frequencies between the two groups. Besides the frequencies for each individual category, we will also compare the frequencies between the two groups for the following grouped categories that are expected side effects of EGCG: 1) GI side effects (Dehydration, Diarrhea, Dyspepsia/heartburn, Nausea, Vomiting, Gastrointestinal-Other) 2) Liver function test abnormalities (Bilirubin, SGOT (AST), SGPT (ALT), Hepatic-Other). We will use Pearson’s Chi-square for adverse events occurring in more than 5 subjects in each group test and Fisher’s exact test for those adverse events with lower frequencies. The DSMB statistician will prepare a similar report for the DSMB meetings.

***Secondary Objectives:***

*Determine if treatment with Polyphenon E alters brain atrophy and peak T1 times, two additional measures of tissue integrity.*

The same mixed model analysis approach as for the primary outcome will be used for the analysis of the peak location and height from the quantitative T1 histograms and the percentage brain volume change.

*Compare the changes in number of new T2 lesions on MRI in MS patients treated with Polyphenon E vs. placebo.*

The number of unique active lesions will be compared using Poisson regression with an effect for treatment and time in the study as the offset variable. Poisson regression is widely used in MS clinical trials for the analysis of this outcome and appropriately deals with the distribution of counts.

*Compare cognitive decay in subjects with MS treated with Polyphenon E vs. placebo*

A MANCOVA model will be fitted using the four primary outcomes as dependent variables. The model will include baseline score on the outcome measures, disease duration and center as covariates. A p value of 0.05 will be considered significant. This will be followed by individual ANCOVA’s with baseline performance on the outcome measure, center and disease duration as covariates. For each of the primary outcome measures a treatment effect will be considered significant at an alpha level of 0.0125 (two sided test) ensuring an overall alpha level of 0.05. Additional baseline covariates that will be considered as predictors for the cognitive tests will be age and education. If any of these baseline covariates are significant predictors of the outcome measure with p value < 0.05 they will be included in the model. Once the model with baseline predictors is considered satisfactory, group allocation will be revealed. The magnitude of the effect will be calculated using the difference in the means adjusted for the covariates included in the model and a 95% CI for the difference will be computed.

*Evaluate changes in disease activity, progression of disability and quality of life in remitting/relapsing MS patients treated with Polyphenon E vs. placebo.*

The number of relapses will be compared using Poisson regression.

Time to sustained progression on the EDSS will be compared using Cox proportional hazards with a main effect for treatment stratified by baseline EDSS. The use of Cox proportional hazards allows for the inclusion of covariates and uses all the available information from all subjects. Cox proportional hazards have been widely used for the analysis of this outcome in MS clinical trials.

Change on the MSFC will be analyzed using ANCOVA on the ranks with an effect for treatment and the presence of enhancing lesions at baseline as a covariate. The distribution of MSFC scores is significantly skewed. ANCOVA on the ranks has been the approach used in prior MS clinical trials for the analysis of this outcome because of its skewed distribution.

A mixed model approach similar to the one used for the primary objective will be used for analyzing the SF-36, PDQ, MSNQ, BDI-II and MFIS.

***Exploratory objectives:***

*Correlate the peak and trough plasma EGCG levels with the changes in NAA levels.*

To evaluate the effect of varying plasma levels on the treatment effect, the analyses of NAA, quantitative T1 and brain atrophy will be repeated replacing the treatment groups with the plasma concentrations as a continuous variable.

*Determine the effects of treatment with Polyphenon E on the rate of change of NAA levels in normal-appearing white matter, grey matter, active lesions and chronic lesions.*

For this objective we will determine the effects of treatment with Polyphenon E on the rate of change of NAA levels in normal appearing white matter, grey matter, active lesions and chronic lesions. For this analysis we will use a mixed model that will include random effects for the slope and intercept for percentages of normal appearing white matter, grey matter (%GM), percentage of active lesion (%AL) and percentage of chronic lesion (%CL) in the voxel and their corresponding fixed effects. The effect of treatment on the change on NAA in each compartment will be evaluated by the treatment\* time\*%NAWM, treatment\* time\*%GM, treatment\* time\*%CL, treatment\*time\*%AL interaction terms.

**Compliance**

To determine the effect of non-compliance on the outcomes we will repeat the analyses on the subset of compliant subjects. Compliant subjects will be defined as those subjects that take at least 75% of the assigned doses.

**Covariates**

Age, gender, presence of enhancing lesions at baseline, MS severity score (percentile rank of EDSS score in a population of people with MS and similar disease duration) , disease type and treatment with GA will be included in all the analyses. Results with and without covariate adjustment will be reported.

**Reporting and Exclusions**

Subjects who are 75% compliant with their medication according to pill counts will be considered fully compliant in terms of efficacy assessments. All data will be used in the analysis, regardless of compliance, but compliance will be investigated as a factor in the analysis.

**Evaluation of Toxicity**

All participants will be evaluable for toxicity from the time of their first dose Polyphenon E.

# ETHICAL AND REGULATORY CONSIDERATIONS

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

**Form FDA 1572**

Prior to initiating this study, the Principal Investigator will provide a signed Form FDA 1572 stating that the study will be conducted in compliance with regulations for clinical investigations and listing the investigators at each site that will participate in the protocol.

**Other Required Documents**

Signed and dated current (within two years) CV or bios ketch for all investigators listed on the Form FDA 1572 for the Lead Organization and all Participating Organizations.

Current medical licenses for all investigators listed on Form FDA 1572 for the Lead Organization and all Participating Organizations.

Lab certification (e.g., CLIA, CAP) and lab normal ranges for all labs listed on Form FDA 1572 for the Lead Organization and all Participating Organizations.

IRB Membership list/letter from IRB for the Lead Organization and all Participating Organizations.

Documentation of training in “Protection of Human Research Subjects” for all investigators listed on the FDA Form 1572 for the Lead Organization and all Participating Organizations.

Documentation of Federal wide Assurance number for the Lead Organization and all Participating Organizations.

Signed receipt of Investigator Brochure

Delegation of Responsibility form

**Institutional Review Board Approval**

Prior to initiating the study and receiving agent, the Principal Investigator must obtain written approval to conduct the study from the appropriate IRB. Should non-administrative changes to the study become necessary, protocol amendments will be approved by the IRB prior to implementation.

**Informed Consent**

All potential study participants will be given a copy of the IRB-approved Informed Consent to review. The investigator will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, he/she will be asked to sign the Informed Consent document. The study agent(s) will not be released to a participant who has not signed the Informed Consent document. Subjects who refuse to participate or who withdraw from the study will be treated without prejudice.

Blood samples will only be used for the research purposes delineated in this protocol.

Any changes to the informed consent document must be submitted to each organization’s IRB for approval prior to implementation.

# REFERENCES

**APPENDICES**

**Appendix A: McDonald Criteria for Multiple Sclerosis Diagnosis**

**Appendix B: Lublin and Reingold Classification for Relapsing/Remitting Multiple Sclerosis**

**Appendix C: Expanded Disability Status Scale (EDSS) Score**

**Appendix D: Data safety and monitoring board charter and data safety and monitoring plan**

**Appendix E: Product information submitted to NIH**

**Appendix F: Investigators brochure**

Appendix G: Questionnaires BDI, PDQ, MSNQ, MFIS, MSFC, SF-36