COVER PAGE

DCP Protocol #: NWU2016-08-02

Local Protocol #: NCI 2016-08-02

A phase I single-arm, multicenter pilot study aimed at validating γ-OHPdG as a biomarker and testing the effects of Polyphenon E® on its levels in patients with cirrhosis.

Consortium Name*: Northwestern Cancer Prevention Consortium
Consortium Principal Investigator (PI): Seema A. Khan, M.D.
Bluhm Family Professor of Cancer Research
Professor of Surgery
Feinberg School of Medicine
303 E. Superior, Suite 4-111
Chicago, IL 60611
Telephone: (312) 503-4236
Fax: (312) 503-2555
E-mail address: skhan@nm.org

Organization Name: Georgetown University
Protocol PI: Aiwu Ruth He, M.D., Ph.D.
Oncology
Lombardi Comprehensive Cancer Center
Georgetown University
3800 Reservoir Road, NW
Washington DC, 20007
Telephone: 202-444-8642
Fax: 202-444-9429
arh29@georgetown.edu

Organization: Georgetown University
Investigator: Fung-Lung Chung, Ph.D.
Departments of Oncology, Biochemistry, and Molecular & Cellular Biology
E215 New Research Building
Washington DC, 20007
Telephone: 202-687-3021
Fax: 202-687-1068
flc6@georgetown.edu

*No participant accrual occurs at this site

Organization: MedStar Health Transplant Institute
MedStar Georgetown University Hospital
Investigator: Coleman Smith, M.D.

MedStar Georgetown University Hospital Transplant Institute
3800 Reservoir Road, NW
Washington DC, 20007

MedStar Georgetown Transplant Institute in Fairfax
3301 Woodburn Road, Suite 209
Annadale, VA 22003
Telephone: 202-444-1085
Fax: 202-444-9429
Coleman.I.Smith@gunet.georgetown.edu

Organization: MedStar Washington Hospital Center
Investigator: Arul Thomas, M.D.
110 Irving St NW
Washington DC 20010
Telephone: 202-444-3700
Arul.M.Thomas@gunet.georgetown.edu

Organization: MedStar Georgetown University Hospital
Investigator: Bhaskar V.S. Kallakury, MD
Department of Pathology
3900 Reservoir Road NW Room SW203, Medical Dental Building Washington, DC 20007
Telephone: 202-687-3444
Fax: 202-687-8935
kallakub@gunet.georgetown.edu

Organization: MedStar Georgetown University Hospital
Investigator: Amol Rangnekar, M.D.
Transplant Institute
3800 Reservoir Rd, NW
Transplant PHC 2nd Floor
Washington, DC 20007
Telephone: 202-444-3700
Amol.S.Rangnekar@gunet.georgetown.edu

Organization: University of Puerto Rico Comprehensive Cancer Center
Investigator: Division of Cancer Biology
Marcia R. Cruz-Correa, M.D., Ph.D., AGAF, FASGE
PO BOX 365067
San Juan, PR 00936
Telephone: 787-772-8300

Fax: 787-758-2557
marcia.cruz1@upr.edu

Organization: University of Puerto Rico Comprehensive Cancer Center
Investigator: Department of Medicine
Victor Carlo, M.D.
PO BOX 365067
San Juan, PR 00936
Telephone: 787-317-3387
Fax: 787-522-3282
victor.carlo@upr.edu

Organization*: Northwestern University
Statistician: Masha Kocherginsky, PhD
Professor of Preventive Medicine
The Feinberg School of Medicine
680 North Lake Shore Drive, Suite 1400
Chicago IL 60611 USA
Telephone: 312.503.4224
Fax: 312.908.9588
mkocherg@northwestern.edu

*No participant accrual occurs at this site

Organization: Georgetown University
Statistician: Hongkun Wang, Ph.D.
Department of Biostatistics, Bioinformatics, and Biomathematics
Building D, Suite 180
4000 Reservoir Road, NW
Washington DC, 20057
Telephone: 202-687-4146
Hongkun.Wang@georgetown.edu

IND Sponsor: NCI/Division of Cancer Prevention
IND# [redacted]
Agent(s)/Supplier: Polyphenon E/EGCG (one capsule of Polyphenon E contains 200mg EGCG) / NCI, Division of Cancer Prevention

NCI Contract # HHSN26122012000351
Protocol Template: Protocol Template Version 8.5 8/31/2016

Protocol Version Date: March 6, 2023

Protocol Revision or Amendment # Version 4.13

SCHEMA

A phase I single-arm, multicenter pilot study aimed at validating γ-OHPdG as a biomarker and testing the effects of Polyphenon E on its levels in patients with cirrhosis.

Participant Flow Schema

Pre-Screen: Chart Review
Medical charts are reviewed by a pathologist for eligibility

↓

Screen 1: Eligibility (n=4-171)
Participants with a diagnosis of liver cirrhosis are seen in clinic:
Informed consent, history/physical/conmed review, CT or MRI, FibroScan®, clinical labs, Child Pugh score, pregnancy test, questionnaires, eligibility review

↓

Screen 2: Liver Biopsy and Eligibility
Participants meeting Screen 1 eligibility criteria are seen in clinic:
Liver biopsy obtained, samples tested for γ-OHPdG level, slides reviewed and scored by a pathologist

↓

Study Visit 1: Week 1 Day 1 (n=2-48)
Participants with high levels of γ-OHPdG (IHC ≥3) meeting eligibility criteria receive Polyphenon E according to the dose escalation schema
3+3 design plus expansion cohort to 24 for Maximum Tolerated Dose:
400 mg EGCG QD
400 mg EGCG BID
600 mg EGCG BID
800 mg EGCG BID
1000 mg EGCG BID
Physical, conmed/AE review, clinical labs, dispense agent, urine/blood collection for PK

↓

Weekly or bi-weekly (depending on assigned dose, clinical stability, and liver biochemical stability of the participant) liver function tests

↓

Study Visit 2: Week 13 ± 3 Days
Mid-study clinic visit:
Physical, conmed/AE review, clinical labs, dispense agent

↓

Weekly or bi-weekly (depending on assigned dose, clinical stability, and liver biochemical stability of the participant) liver function tests

↓

Study Visit 3: Week 25 ± 3 Days
Last day of agent:
Physical exam, conmen/AE review, clinical labs, Child Pugh score, CT or MRI, FibroScan, liver biopsy testing for γ-OHPdG

↓

Study Visit 4: Week 28 ± 3 Days
Follow-up:
Physical exam, clinical labs

Primary Objectives:

- To establish maximum tolerated dose (MTD) and to collect safety data of Polyphenon E/EGCG treatment in patients with cirrhosis.
- To determine the effects of Polyphenon E/EGCG treatment on the suppression of γ-hydroxy-1,N(2)-propanodeoxyguanosine (γ-OHPdG) levels in cirrhotic liver.

Secondary Objectives:

- To collect Polyphenon E/EGCG pharmacokinetic data in patients with cirrhosis.
- To determine the effects of Polyphenon E/EGCG treatment on the suppression of γ-hydroxy-1,N(2)-propanodeoxyguanosine (γ-OHPdG) levels in cirrhotic liver by LC-MS assay from baseline to post-treatment.
- To estimate the fraction of patients with liver cirrhosis that have high levels of γ-OHPdG.

Exploratory Objective:

- To assess the effects of Polyphenon E/EGCG on the grade of cirrhosis as measured by FibroScan and FIB-4 score.
- To develop an LC-MS and/or ELISA-based method for detecting urinary and blood γ-OHPdG, to correlate with liver γ-OHPdG levels.
- To evaluate any HCC development during the treatment.

TABLE OF CONTENTS

COVER PAGE 1
SCHEMA 4
1. OBJECTIVES 7
1.1 Primary Objective 7
1.2 Secondary Objectives 7
1.3 Exploratory Endpoint 7
2. BACKGROUND 7
2.1 Hepatocellular carcinoma (HCC) 7
2.2 Cirrhosis 7
2.3 γ-OHPdG, the biomarker to be tested in this protocol 9
2.4 Polyphenon E 16
2.5 Rationale 17
3. SUMMARY OF STUDY PLAN 20
4. PARTICIPANT SELECTION 22
4.1 Inclusion Criteria 22
4.2 Exclusion Criteria 24
4.3 Inclusion of Women and Minorities 25
4.4 Recruitment and Retention Plan 25
5. AGENT ADMINISTRATION 26
5.1 Dose Regimen and Dose Groups 26
5.2 Polyphenon E Administration 27
5.3 Run-in Procedures 27
5.4 Contraindications 28
5.5 Concomitant Medications 28
5.6 Dose Interruption and Modification 28
5.7 Adherence/Compliance 29
6. PHARMACEUTICAL INFORMATION 29
6.1 Polyphenon E (IND [redacted], NCI/DCP) 29
6.2 Reported Adverse Events and Potential Risks 30
6.3 Availability 32
6.4 Agent Distribution 32
6.5 Agent Accountability 33
6.6 Packaging and Labeling 33
6.8 Registration/Randomization 33
6.9 Blinding and Unblinding Methods 34
6.10 Agent Destruction/Disposal 34
7. CLINICAL EVALUATIONS AND PROCEDURES 35
7.1 Schedule of Events 35
7.2 Baseline Testing/Prestudy Evaluation 37
7.3 Evaluation During Study Intervention 38
7.4 Evaluation at Completion of Study Intervention 41
7.5 Post-intervention Follow-up Period/ Final Evaluations, Off Study 41
7.6 Methods for Clinical Procedures 42
8. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION 45
8.1 Primary Endpoint 45
8.2 Secondary Endpoints 46
8.3 Off-Agent Criteria 47
8.4 Off-Study Criteria 48
8.5 Study Termination 48
9. CORRELATIVE/SPECIAL STUDIES 49
9.1 Rationale for Methodology Selection 49

9.2 Comparable Methods 50
10. SPECIMEN MANAGEMENT 51
10.1 Laboratories 51
10.2 Collection and Handling Procedures 51
10.3 Shipping Instructions 55
10.4 Tissue Banking 56
11. REPORTING ADVERSE EVENTS 56
11.1 Adverse Events 56
11.2 Serious Adverse Events 58
12. STUDY MONITORING 59
12.1 Data Management 59
12.2 Case Report Forms 60
12.3 Source Documents 60
12.4 Data and Safety Monitoring Plan 60
12.5 Sponsor or FDA Monitoring 60
12.6 Record Retention 60
12.7 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA) 61
13. STATISTICAL CONSIDERATIONS 61
13.1 Study Design/Description 61
13.2 Randomization/Stratification 61
13.3 Accrual and Feasibility 62
13.4 Primary Objective, Endpoint(s), Analysis Plan 63
13.5 Secondary Objectives, Endpoints, Analysis Plans 64
13.6 Reporting and Exclusions 65
13.7 Evaluation of Toxicity 65
13.8 Evaluation of Response 66
13.9 Interim Analysis 66
13.10 Ancillary Studies 66
14. ETHICAL AND REGULATORY CONSIDERATIONS 66
14.1 Form FDA 1572 66
14.2 Other Required Documents 66
14.3 Institutional Review Board Approval 67
14.4 Informed Consent 67
14.5 Submission of Regulatory Documents 67
14.6 Other 68
15. FINANCING, EXPENSES, AND/OR INSURANCE 68
REFERENCES 69
Consent Form 74
APPENDIX A 88
APPENDIX B 89
APPENDIX C 90
APPENDIX D 91
APPENDIX E 95
APPENDIX F 97
APPENDIX G 100
APPENDIX H 101
APPENDIX I 104

1. OBJECTIVES

1.1 Primary Objective

1.1.1 To establish maximum tolerated dose (MTD) and to collect safety data of Polyphenon E/EGCG treatment in participants with cirrhosis.

1.1.2 To determine the effects of Polyphenon E/EGCG treatment on the suppression of γ-hydroxy-1,N(2)-propanodeoxyguanosine (γ-OHPdG) levels in cirrhotic liver.

1.2 Secondary Objectives

1.2.1 To collect Polyphenon E/EGCG pharmacokinetic data in participants with cirrhosis.

1.2.2 To determine the effects of Polyphenon E/EGCG treatment on the suppression of γ-hydroxy-1,N(2)-propanodeoxyguanosine (γ-OHPdG) levels in cirrhotic liver by LC-MS assay from baseline to post-treatment.

1.2.3 To estimate the fraction of participants with liver cirrhosis that have high levels of γ-OHPdG.

1.3 Exploratory Endpoint

1.3.1 To assess the effects of Polyphenon E/EGCG on the grade of cirrhosis as measured by FibroScan® and FIB-4 score.

1.3.2 To develop an LC-MS and/or ELISA-based method for detecting urinary and blood γ-OHPdG, to correlate with liver ɣ-OHPdG levels.

1.3.3 To evaluate any HCC development during the treatment.

2. BACKGROUND

2.1 Hepatocellular carcinoma (HCC)

HCC is the second and sixth leading cause of cancer related mortality in men and women, respectively (1). Despite advances in our understanding of the molecular pathways involved in HCC, our therapeutic armamentarium remains limited and survival dismal. In early stage disease (Barcelona clinic liver cancer [BCLC] stages 0 and A), surgical resection, ablation methods, and liver transplantation reportedly extend survival by more than 5 years. However, less than half of participants have resectable tumors at initial presentation and, even after successful resection, 70% will eventually relapse due to micrometastases and de-novo malignant transformation of adjacent liver cells (2,3). There is no preventive treatment for this deadly disease at this time.

2.2 Cirrhosis

2.2.1 Diagnosis of cirrhosis.

Despite geographical differences, chronic liver diseases are highly prevalent worldwide. It is estimated that at least 350 million and 120 million people globally are chronically infected with hepatitis B virus (HBV) and hepatitis C virus, respectively. (4,5) Non-alcoholic fatty liver disease (NAFLD) affects 15-40% of the general population and is particularly prevalent in participants with diabetes and obesity.(6-8) Alcoholic liver disease affects people in both developed and developing countries and may account for up to 9.2% of all disability-adjusted life years in some regions. (9) Although the etiologies are different, chronic liver disease leads to liver injury, progressive liver fibrosis, and finally to the stage of cirrhosis and liver decompensation. As a result, cirrhosis remains the twelfth global leading cause of death in 2010. (10)

The diagnosis of cirrhosis is not as simple as it seems. It is straightforward when a patient has already developed clinical manifestations of portal hypertension such as ascites, varices and hypersplenism. However, these signs are absent in participants with early cirrhosis, and the radiological features of early cirrhosis are subtle and unreliable. (11) Cirrhosis is usually diagnosed by way of liver biopsy, which is an invasive procedure with a small risk of bleeding. In recent years, the development and application of non-invasive tests of liver fibrosis have revolutionized hepatology practice. Numerous studies have confirmed the accuracy of FibroScan in fibrosis staging and the diagnosis of cirrhosis. In general, the tests have high negative predictive value for the exclusion of advanced fibrosis and cirrhosis, and have been recommended by the European Association for the Study of the Liver as initial assessment in participants with various liver diseases. (12)

The Fibrosis-4 (FIB-4) score is an exploratory endpoint which helps to estimate the amount of scarring in the liver (see the equation below). Using a lower cutoff value of 1.45, a FIB-4 score < 1.45 had a negative predictive value of 90% for advanced fibrosis (Ishak fibrosis score 4-6 which includes early bridging fibrosis to cirrhosis). In contrast, a FIB-4 > 3.25 would have a 97% specificity and a positive predictive value of 65% for advanced fibrosis.

$$\text{FIB-4} = \frac{\text{Age (years)} \times \text{AST (U/L)}}{\text{Platelet Count } (10^9/\text{L}) \times \sqrt{\text{ALT (U/L)}}}$$

2.2.2 Compensated vs. Decompensated cirrhosis

Cirrhosis is the end-stage of every chronic liver disease; that is, if participants don't go on to develop HCC. Its natural history is characterized by an asymptomatic phase, termed 'compensated' cirrhosis followed by a rapidly progressive phase marked by the development of complications of portal hypertension and/or liver dysfunction, termed 'decompensated cirrhosis'. In the compensated phase, portal pressure may be normal or below the threshold level identified for the development of varices or ascites ('clinically significant portal hypertension'). (13,14) As the disease progresses, portal pressure increases and liver function decreases, resulting in the development of ascites, portal hypertensive gastrointestinal (GI) bleeding, encephalopathy, and jaundice. The development of any of these complications marks the transition from a compensated to a decompensated phase.

2.2.3 Risk of development of HCC.

Participants with liver cirrhosis, independent of their etiology, as well as individuals without cirrhosis but with chronic hepatitis B infection or non-alcoholic fatty liver disease (NAFLD) have a very high risk for the development of hepatocellular carcinoma (HCC). (15) Worldwide, HCC is the fifth most common malignancy. (57) In Europe, North America, and Japan, the HCC incidence is lower compared to South-Asian countries and Africa, but not negligible. (58) Thus, a challenging task is the early diagnosis of HCC in participants with known risk factors. Participants with HCC and tumor symptoms have a survival rate of only 0-10%. (59) In contrast, participants with an early diagnosis of HCC can achieve 5-year survival rates of over 50%. (15) The surveillance for HCC in participants with cirrhosis by clinic visit, laboratory test, and twice per year scan is recommended by AASLD. However, the current best prevention strategy for HCC development is to avoid the development of liver disease and the progression to liver cirrhosis. Effective HCC preventive interventions in participants with cirrhosis are lacking.

2.3 γ-OHPdG, the biomarker to be tested in this protocol

2.3.1 γ-OHPdG is a lipid peroxidation (LPO)-derived endogenous cyclic DNA adduct, which causes somatic mutations and is a possible surrogate marker for inflammation-associated cancer. It is known that chronic inflammation leads to oxidative/nitrosative stress and lipid peroxidation (LPO), generating excess reactive oxygen species (ROS) and reactive nitrogen species (RNS) (1-19). Highly reactive α, β-unsaturated aldehydes (enals) are also generated due to oxidation of membrane polyunsaturated fatty acids (PUFAs), which can modify DNA bases, forming promutagenic cyclic DNA adducts (20-23). Oxidative stress caused by chronic inflammation has emerged as a major player in liver carcinogenesis of different etiologies, including HCV/HBV, alcoholism, and obesity (24). Oxidative stress drives genomic damage and genetic instability to cause mutations. LPO-derived endogenous cyclic DNA adducts have been investigated as potential markers for various types of inflammatory cancer-prone diseases (e.g. chronic pancreatitis, Crohn`s disease, ulcerative colitis, alcohol related hepatitis, and H. pylori infection) and cancer initiation/promotion (25). Among the LPO-derived DNA adducts, γ-hydroxy-1,N(2)-propanodeoxyguanosine (γ-OHPdG) is one of the most abundant and well-studied adducts, and has been ubiquitously detected in mammalian tissues (26). γ-OHPdG is formed due to modification of deoxyguanosine (dG) in DNA by acrolein, an aldehyde that is a major constituent of cigarette smoke, as well as being endogenously formed by LPO. The γ-OHPdG adduct is mutagenic and induces predominantly G to T and G to A mutations (27-30). One study found that γ-OHPdG occurs at sites that coincide with p53 mutation hotspots in lung cancers of smokers (31). These results support the role of γ-OHPdG-induced somatic mutations in human cancers.

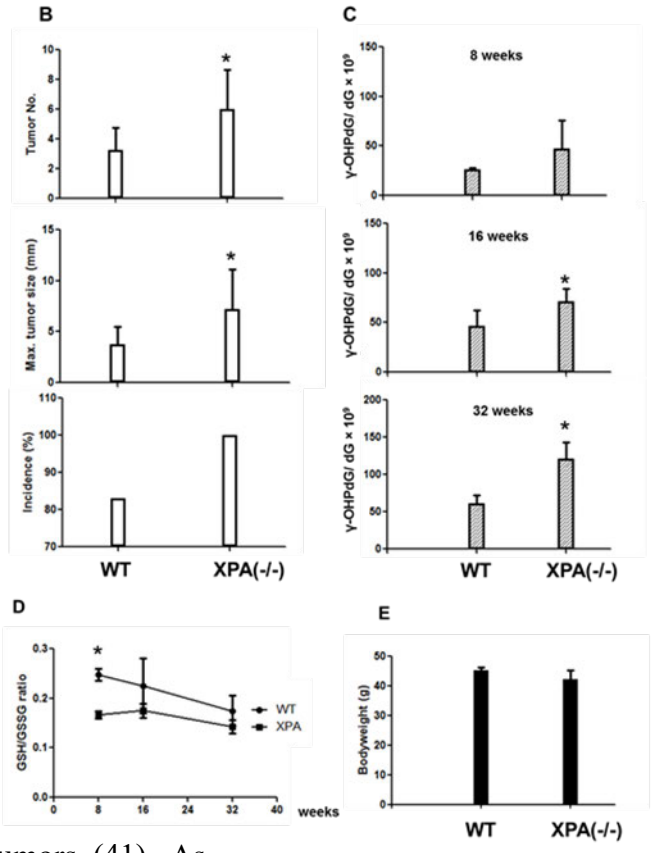
Epidemiological studies have shown antioxidants to be preventive against various types of cancer (32); however, contradicting results have been obtained in intervention trials (33, 34). A possible explanation for the contradictory results is bias from unmeasured confounders in the epidemiological studies. Biomarkers are crucial for the selection of participants likely to benefit from antioxidant therapy. There is thus an unmet and urgent need for the detection and validation of carcinogenesis-related biomarkers in cancer prevention trials. The prevalence of a commonly oxidized DNA base—8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxo-dG)—has been found to increase during hepatocarcinogenesis (35, 36). However, to date there have been no reports of

specific mechanism-based DNA adducts acting successfully as predictive biomarkers for hepatocellular carcinoma (HCC).

Ongoing studies in our laboratory are focused on the role of γ-OHPdG in hepatocarcinogenesis, as well as elucidation of the mechanism by which Theaphenon® E/Polyphenon E prevent accumulation of γ-OHPdG, and thus HCC.

2.3.2 γ-OHPdG is a biomarker of hepatocarcinogenesis in animals and correlates with high levels of GC to TA and GC to AT mutations

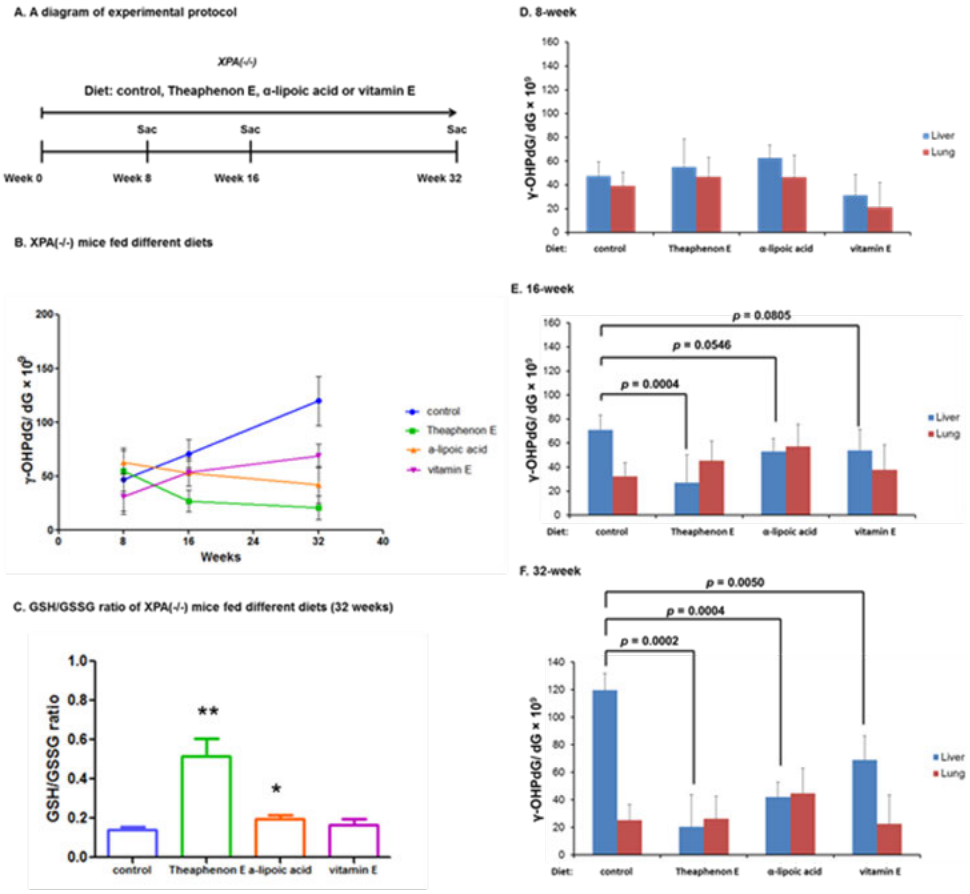
Figure 1



Primary liver cancer is the third most common cause of cancer-related death globally; this disease lacks a suitable biomarker for early detection, is resistant to current chemotherapy, and has molecular features that are inadequately understood (37). HCC accounts for approximately 85% of liver cancer and is an inflammation-associated cancer (38, 39). The mutagenicity of γ-OHPdG has been well studied in vitro (40) but in vivo data is lacking. In our laboratory, we first studied γ-OHPdG as a biomarker of hepatocarcinogenesis in vivo in C57/B6 mice (transgenic Xpa-/- and diethylnitrosamine (DEN)-induced HCC models) and rats (Long Evans Cinnamon [LEC]).

Xpa-/- C57/B6 mice are primarily used as a skin-cancer model following exposure to ultraviolet (UV)-B irradiation; however, these mice also develop spontaneous liver tumors (41). As repair of γ-OHPdG DNA adducts occurs via nucleotide excision repair (NER) (42), and the xeroderma pigmentosum group A correcting (Xpa) protein participates in the initial steps of NER, we proposed that an accumulation of γ-OHPdG in Xpa-/- mice occurs due to lack of NER activity, and this contributes to the observed hepatocarcinogenesis in this model. We back-crossed Xpa-/- C57/B6 mice with C3H/HeNCrl mice in order to breed mice with a high rate of spontaneous liver cancer (43). Compared with WT controls, these Xpa-/- mice not only had a higher incidence of liver tumors, but also showed significantly larger tumor sizes and increased multiplicity (fig. 1). The γ-OHPdG levels increased age-dependently in Xpa-/- mice from age 8-week to 32-week ($p < 0.05$, fig. 1C). We were able to detect a statistically significant difference in the hepatic levels of γ-OHPdG and the liver cancer development between Xpa-/- and WT mice (fig. 1).

Figure 2



The relationship of γ-OHPdG in HCC was further examined in C57/B6 mice involving a single injection of procarcinogen DEN (fig 2). In this model, mice develop poorly differentiated HCC nodules within 32 to 36 weeks after DEN exposure (44). We administered DEN (5 mg/kg) to male mice on postnatal day 14, and placed the mice on AIN-76A powder diet. A time-dependent increase of steatosis was observed in the mice under the control diet. HCCs were observed after 6 months. An age-dependent increase of γ-OHPdG was seen in the livers, but not in the lungs (a non-target tissue). These results are consistent with previous reports that DEN can cause oxidative stress and induce LPO (45). LEC rats are inflicted with increased LPO due to abnormal copper accumulation, mimicking that of human Wilson's disease (46). As a result, LEC rats develop acute hepatitis, followed by chronic hepatitis, and eventually HCC (39). We found that γ-OHPdG levels in the livers of LEC rats were significantly higher than those seen in WT Long Evans (LE) rats (data not shown). These results provide additional support for elevated γ-OHPdG levels in the livers of animals with an increased risk of HCC.

Studies have shown that γ-OHPdG causes GC to TA and GC to AT mutations (27, 28). We reasoned that the increased γ-OHPdG in the livers of Xpa-/- mice can lead to a somatic mutation pattern in which GC to TA and GC to AT mutations are the most frequent alterations. We compared mutation frequencies in 2 pairs of liver tumor nodules versus adjacent normal liver tissues from 2 Xpa-/- mice using whole exome next-generation sequencing (NGS). Whole exome sequencing produced a mean yield of 23.8 million reads or 2.5 gigabases of data per sample with 94.1% > Q30. The samples were sequenced to a mean coverage of 29X, and 99.4% of the reads were mapped to the target regions. We found 60 and 100 variants in the 2 liver nodules, with GC to TA mutation as the dominant alteration, accounting for 92% and 86% mutations, respectively. While examining the Sorting Intolerant from Tolerant (SIFT) prediction scores, we also noted that

more than 35% of the variants in both samples were predicted to be deleterious mutations. The high GC to TA mutation frequency in the Xpa-/- mouse liver cancers suggests that γ-OHPdG plays a role in the mutagenesis that leads to HCC development. This high GC to TA mutation frequency is also different from other solid tumors in which CG to TA transitions are the highest variations in the mutation spectrum. Variants in human HCC also showed an over-representation of GC to TA transversion (41). We identified a number of mutant genes within mouse liver nodules that were also reported in human HCC, including ABCA1, CSMD1, LAMA2, TRRAP, and TRANK1 (19), suggesting that the Xpa-/- mouse model is relevant to human liver carcinogenesis.

2.3.3 Antioxidants suppress liver γ-OHPdG levels and HCC development in Xpa-/- mice.

To further assess the role of γ-OHPdG in HCC development, we determined if blockage of γ-OHPdG formation in the livers of Xpa-/- mice would reduce HCC incidence. Xpa-/- mice had 100% liver cancer incidence at the end of our 72-week bioassay. Three antioxidants known to suppress LPO—Theaphenon E (a similar green tea polyphenol to Polyphenon E), α-lipoic acid, and vitamin E—were used (47). To determine whether these antioxidants suppressed liver γ-OHPdG levels, Xpa-/- mice were fed diets containing them. We observed a significant decrease in liver γ-OHPdG levels in the mice fed all 3 antioxidant diets, although they appeared to have different potencies: Theaphenon E > α-lipoic acid > vitamin E (fig. 3). However, no significant changes in γ-OHPdG levels in lung, a non-target organ, were found. We also examined the ratio of reduced glutathione to oxidized glutathione (GSH:GSSG), an indicator of oxidative stress. The increases observed in the GSH:GSSG ratio in the liver tissues from mice fed different antioxidant diets for 32 weeks were consistent with the decreases in γ-OHPdG levels (fig. 3).

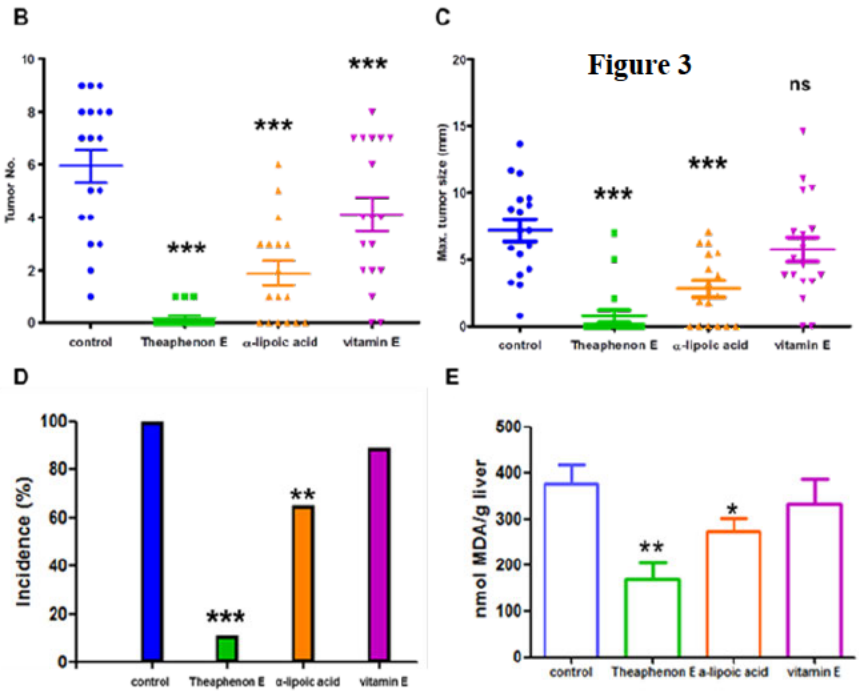


Figure 3

Having demonstrated that antioxidants can suppress γ-OHPdG formation, we next examined the relationships between γ-OHPdG and hepatocarcinogenesis. Xpa-/- mice were fed 4 different diets for 72 weeks: Theaphenon E-, α-lipoic acid-, or vitamin E-containing, or control diets. Hepatocarcinogenesis was most strongly reduced in mice fed Theaphenon E (14% HCC incidence vs. 100% in mice fed the control diet). α-Lipoic acid resulted in a decrease of HCC incidence to 65%, whereas vitamin E had no significant effect (fig. 3). The potency of tumor inhibition by the antioxidants showed a strong correlation with the liver γ-OHPdG levels in these mice. The protective effects of antioxidants against HCC were similar in both male and female mice (data

not shown), which is in agreement with the effects on γ-OHPdG suppression in both genders. The potency of LPO inhibition by the antioxidants, obtained by measuring MDA levels in mouse livers, is consistent with that of γ-OHPdG (fig. 3E). Although no food consumption differences were observed, mice in the Theaphenon E group weighed less (were leaner) and appeared healthier than mice in the other groups. This is probably related to thermogenesis, fat oxidation, and the sparing of fat free mass effects known to be caused by green tea extract (48).

We tested whether antioxidants can suppress HCC development in the DEN mouse model. Similar to Xpa-/- mouse results, Theaphenon E showed remarkable suppression of HCC formation in these mice, decreasing incidence from 100% to 40%, tumor size from 10 mm to < 1 mm, and multiplicity from 30 to 3 nodules per mouse. Again, Theaphenon E effectively inhibited HCC formation in the DEN-induced HCC model.

2.3.4 γ-OHPdG is elevated in human cirrhotic liver compared with normal liver.

The data described above demonstrates that γ-OHPdG is closely associated with liver carcinogenesis in animal models. To investigate the role of γ-OHPdG in human HCC, 40 human liver samples were procured with different pathologies/diagnoses, including 2 normal livers, 7 livers with cirrhosis, 4 with hyperplasia, 3 with cirrhosis and hyperplasia, and 22 with HCC. Samples were stained with a γ-OHPdG monoclonal antibody developed in our laboratory (49). The association between pathology and expression level of γ-OHPdG was examined using Fisher test. A significant association was found between liver disease progression and the immunoscore of γ-OHPdG with $p = 0.0364$. However, the immunoscore of γ-OHPdG was not associated with the stages of HCC ($p = 0.3259$).

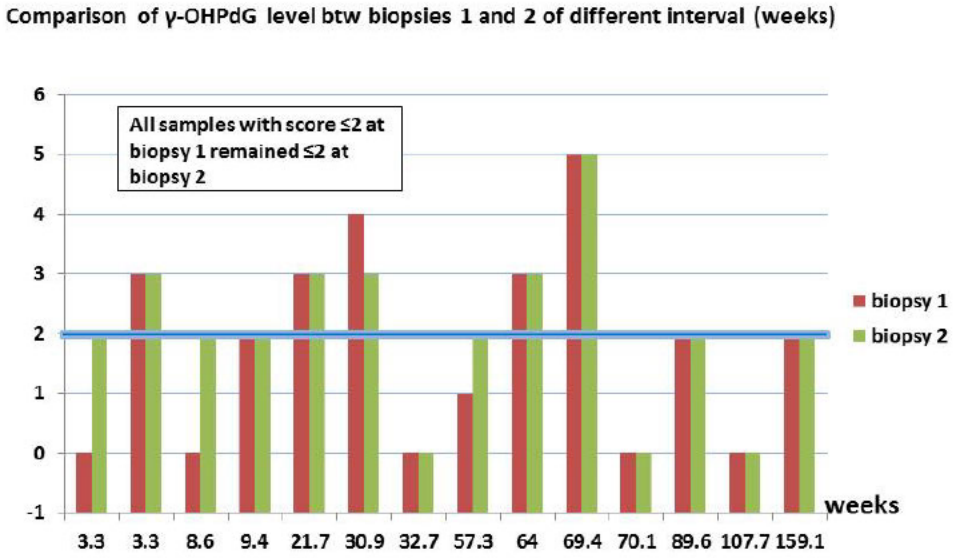
We then further characterized γ-OHPdG adduct levels in participants with cirrhosis (an advanced stage of liver disease preceding HCC development). Sixty-eight human liver biopsy samples from participants with liver cirrhosis but without HCC at the time of biopsy were stained with a γ-OHPdG monoclonal antibody. γ-OHPdG levels were scored on a scale of 0-3 for distribution of positive stain, and again, 0-3 for intensity of positive stain. Sixty-two percent (n = 42) had an IHC score of 2 or less, and 38% (n = 26) had an IHC score of 3 or higher (Table 1).

2.3.5 Stability of γ-OHPdG levels in each patient's cirrhotic liver over time

In order to evaluate γ-OHPdG as a biomarker for HCC development in cirrhotic participants, the stability of the biomarker was studied in participants with serial liver biopsies. The levels of γ-OHPdG were compared between the 1st and 2nd biopsies in 20 cirrhotic participants with serial liver biopsies (fig 4). Six of the 20 participants were not suitable for analysis: 2 subjects (cases 4 and 6) had liver transplantation between the 2 biopsies; 2 (cases 13 and 22) did not have cirrhosis at the first biopsy, but had developed cirrhosis at the second biopsy; 2 (cases 20 and 23) had developed HCC between the first and second liver biopsies. Once these 6 cases had been excluded from the study, the remaining 14 subjects were evaluated for the stability of γ-OHPdG in their serial biopsies. The median interval between the 2 serial biopsies was 7 months (range: 1-37 months). The γ-OHPdG levels from all 14 subjects remained in either the low IHC score category (2 or less) or the high IHC score category (3 or higher), i.e., there was no crossing over from low to high or high to low in the interval between the 2 biopsies.

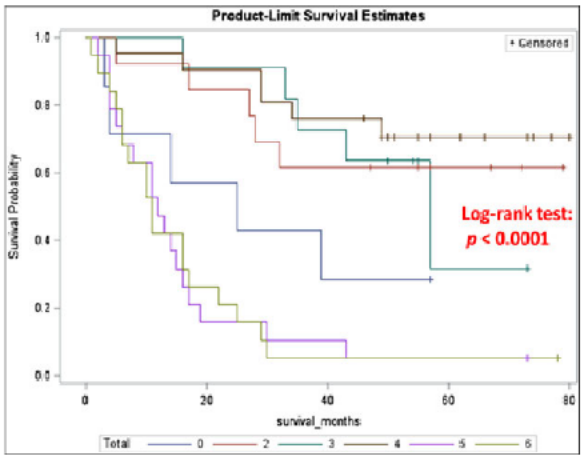
2.3.6 γ-OHPdG predicts human HCC recurrence and survival after curative resection.

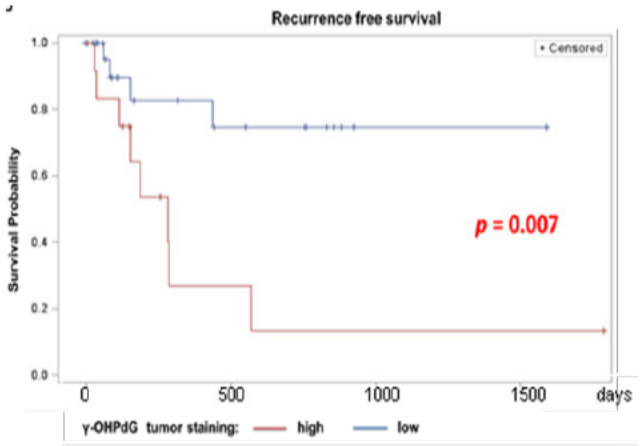
Figure 4



To determine whether γ-OHPdG serves as a predictive biomarker for the survival of HCC participants, we identified liver samples from 2 independent sets of HCC participants who underwent surgery. The first set composed of ninety HCC participants who underwent surgery without adjuvant therapy between August 2006 and November 2009. These participants were followed up for 4-7 years. We found that the high γ-OHPdG levels in the tumor were strongly associated with poorer survival in these participants ($p < 0.0001$). We then recruited 45 participants and attempted to correlate their liver γ-OHPdG levels with their recurrence-free survival. Participants with low γ-OHPdG tumors (IHC score ≤ 3) experienced a significantly prolonged HCC recurrence-free survival compared with participants with high γ-OHPdG tumors (IHC sore > 3) ($p = 0.007$, fig. 5). After 2 years of follow-up from the date of curative surgical resection, the probability of no cancer recurrence in participants with low γ-OHPdG tumors was 75%, while the probability of no cancer recurrence in participants with high γ-OHPdG tumors was only 13% ($p = 0.0002$). These clinical studies provide validation for the use of γ-OHPdG as a biomarker for the prediction of risk of human HCC recurrence.

Figure 5





In summary, the use of γ-OHPdG, a LPO-derived endogenous mutagenic DNA adduct, as a mechanism-based prognostic biomarker for HCC development is supported by data from 3 HCC animal models. Theaphenon E, an antioxidant formulation from green tea extract, effectively

suppressed the accumulation of γ-OHPdG and inhibited HCC formation in animal models of HCC. We reported that γ-OHPdG is elevated in a portion of human cirrhotic liver, and found that the levels of γ-OHPdG remain relatively stable in cirrhotic participants over time. Furthermore, γ-OHPdG serves as a biomarker of HCC recurrence. The study revealed that higher levels of γ-OHPdG are strongly associated with low recurrence-free survival ($p < 0.007$). Collectively, these results support the role of γ-OHPdG in HCC development and provide a strong rationale for the development of an antioxidant formulation of green tea extract (i.e., Theaphenon E/Polyphenon E) for HCC prevention. To date, no chemopreventive agents have been developed for liver cancer. Although Polyphenon E has been studied in other cancers, it has never been examined in the prevention of HCC. This study will verify the role of γ-OHPdG as a predictive biomarker of HCC; provide a novel prevention strategy for liver cancer, the third leading cause of cancer-related death globally; and expand knowledge of the antioxidant effects of green tea polyphenols in the prevention of liver cancer.

2.3.7 Alcohol and Tobacco Use Questionnaires

Increasing evidence suggests that tobacco and alcohol use are risk factors in the development of intraepithelial neoplasia and cancer. In addition, tobacco and alcohol use may adversely affect agent intervention, for example by altering the safety profile or metabolism of a drug. Standardized assessments of tobacco and alcohol use during clinical trials will aid in understanding the potential relationship between the use of these products and clinical endpoints or cancer prevention biomarkers. Therefore, NCI, DCP is including assessment of tobacco and alcohol use at baseline and Study Visit 3, to determine the potential impact of tobacco and alcohol use on 1) treatment toxicity and symptom burden, and 2) the efficacy of treatment intervention.

2.4 Polyphenon E

Green tea has been shown to exhibit cancer-preventive activities in preclinical studies. Its principal active components include epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin (EC), and epicatechin gallate (EG), of which EGCG is the most abundant and possesses the most potent antioxidant activity. Theaphenon E and Polyphenon E have the same chemical composition. Polyphenon E formulation has been granted an IND by the FDA and is produced for human studies.

2.4.1 Pharmacokinetics of Polyphenon E

A Phase I pharmacokinetic study was carried out to determine the systemic availability of green tea catechins after single oral dose administration of Polyphenon E (decaffeinated green tea catechin mixture) (50). Twenty healthy subjects (5 subjects/ per dose level) were randomly assigned to one of the dose levels (200, 400, 600, and 800 mg based on EGCG content). The AUC and Cmax of EGCG after the 800- mg dose were significantly higher than those after the 3 lower doses. EGC and EC were present in the body after the Polyphenon E administration; however, they were present predominantly in their conjugated forms. The effect of food on oral bioavailability of Polyphenon E administration was tested in 30 healthy volunteers, and it was found that taking the Polyphenon E capsules on an empty stomach resulted in greater oral bioavailability of free catechins (51, 52). The pharmacokinetics of the conjugated metabolites of EGC and EC were not affected by repeat green tea polyphenol daily treatment for 4 weeks. A limited sampling strategy

was developed to predict EGCG pharmacokinetics after green tea administration (53). The median sampling times were 0.7, 1.4, and 7.0 hours (fasting conditions) and 1.4, 3.6, and 8.7 hours (fed conditions). The sampling schemes were accurate and precise in predicting EGCG oral clearance and exposure (area under the curve [AUC]) under both fasting and fed conditions. In our study, participants who agree to do the PK tests at Georgetown will be asked to fast on their first study visit and blood and urine samples will be collected on day 1 of a 4 week cycle, prior to the first dose, and then at 1.5, 3.5, and 8.5 hours (+/- 5 minutes) after the first dose of Polyphenon E. Clearance of Polyphenon E will be compared among cirrhotic liver participants in this study and results from this population will be compared with non-cirrhotic historical control participants (53).

2.4.2 Supply of Polyphenon E for this study

The Chemopreventive Agent Development Research Group, Division of Cancer Prevention, National Cancer Institute, NIH is supporting this clinical study by providing Polyphenon E as 200 mg capsules. The capsules will provide 200 mg of EGCG each. Participants will take 2 - 5 capsules once or twice per day continuously for six months, according to the dose escalation 3 + 3 design. Polyphenon E/EGCG capsules will be stored at room temperature, avoiding direct sun exposure.

2.5 Rationale

2.5.1 Overall Rationale

γ-Hydroxy-1, N2-propanodeoxyguanosine (γ-OHPdG) is a lipid peroxidation-derived mutagenic DNA adduct, which is repaired by the nucleotide excision repair (NER) pathway. Xeroderma pigmentosum group A (Xpa) knockout mice deficient in NER developed a high incidence of spontaneous liver tumors with more than 85% of GC to TA somatic mutations. We found that levels of γ-OHPdG showed an age-dependent increase in mice, and that they were higher in the livers of Xpa-/- mice than WT mice. Theaphenon E, an anti-oxidant, effectively decreased γ-OHPdG levels in the liver DNA of Xpa-/- mice and reduced HCC incidence to 14% and 65%, respectively, from 100% in the untreated controls. γ-OHPdG was also found to be elevated in the livers of diethyl nitrosamine (DEN)-treated mice and Long-Evans Cinnamon rats—other models with high risk of HCC. Again, Theaphenon E effectively inhibited HCC formation in the DEN-induced HCC model. We examined whether γ-OHPdG serves as a biomarker of HCC recurrence using HCC specimens from 45 participants. The study revealed that higher levels of γ-OHPdG are strongly associated with low recurrence-free survival ($p < 0.0002$). Together, these results suggest γ-OHPdG is a potentially biologically relevant biomarker for predicting the risk of HCC.

Three antioxidants known to suppress LPO—Theaphenon E (a similar green tea polyphenol to Polyphenon E), α-lipoic acid, and vitamin E—were used (47). We observed a significant decrease in liver γ-OHPdG levels in the mice fed all 3 antioxidant diets, although the individual antioxidants appeared to have different potencies: Theaphenon E > α-lipoic acid > vitamin E (fig 2 and fig. 3). Theaphenon E, α-lipoic acid, and vitamin E decreased the accumulation of γ-OHPdG levels by 65%, 30%, and 30%, respectively, after 16 weeks of treatment, and 83%, 66% or 50%, respectively, after 32 weeks of treatment. We next examined the relationship between γ-OHPdG and hepatocarcinogenesis in the 3 treatment groups. Hepatocarcinogenesis was most strongly reduced in mice fed Theaphenon E (14% HCC incidence vs. 100% in mice fed the control diet), α-Lipoic acid resulted in a decrease of HCC incidence to 65%, whereas vitamin E had no significant effect (fig. 3). The potency of tumor inhibition by the antioxidants showed a strong

correlation with liver γ-OHPdG levels in treated mice. In our preclinical model, Theaphenon E/Polyphenon E appeared to be superior in its suppression of γ-OHPdG accumulation and HCC development in comparison to α-lipoic acid or vitamin E. α-Lipoic acid has been mainly tested in cardiovascular diseases (CVD), obesity, pain, inflammatory diseases, and aging (54) and was found to be a reasonable alternative to Polyphenon E, although α-lipoic acid is not as potent as Polyphenon E in the prevention of liver cancer in our preclinical HCC models.

We hypothesize that accumulation of the DNA adduct, γ-OHPdG, predicts the development of HCC, and that the dietary antioxidant, Theaphenon E/Polyphenon E, suppresses the formation of γ-OHPdG, which may reduce HCC development in participants with cirrhosis.

HYPOTHESES: 1) accumulation of γ-OHPdG predicts the development of HCC in cirrhotic participants. 2) Polyphenon E suppresses the formation of γ-OHPdG and reduces HCC development in participants with cirrhosis.

2.5.2 Polyphenon E Dose Selection /Modification

In previous chemoprevention trials, the daily dose of Polyphenon E ranged from 400 to 4000 mg EGCG. It is not known whether the maximum tolerated dose (MTD) of Polyphenon E/EGCG is actually needed to bring about the greatest possible reduction of γ-OHPdG in cirrhotic liver. To ensure the safe use of Polyphenon E in cirrhotic participants, we propose a 3+3 dose escalation study with daily EGCG dose levels of 400 mg, 800 mg, 1200 mg, 1600 mg, and 2000 mg, with an expansion cohort such that a total of 24 subjects complete the study at the MTD as shown in the study scheme on page 4 of the protocol. MTD is defined as the dose at which ≤1 subjects out of 6 experiences a Grade 3 or higher toxicity based on CTCAE criteria, or requires discontinuation of treatment based on the Hepatotoxicity Assessment Algorithm provided in the Appendix (shown in Appendix D). Subjects at one dose level will have liver function & coagulation tests repeated every 2 weeks; if no subject requires discontinuation of treatment during the first 4 weeks of treatment based on the Hepatotoxicity Assessment Algorithm, subjects will then be enrolled at the next dose level. With consideration of the underlying liver cirrhosis in our study population, we propose a dose escalation study with careful safety monitoring for liver toxicities.

The maximum dose of 2000 mg EGCG daily selected for the dose escalation cohort is only 50% of the maximum dose used in a previous phase II study [56]. This study design will ensure safety of cirrhotic participants, and provide an opportunity to evaluate the effect of EGCG on γ-OHPdG at a different (lower) dose level.

EGCG

One published review assessed 34 clinical trials that evaluated green tea extracts in which EGCG was the main component (57). The summary odds ratio, estimated using a meta-analysis method for sparse event data, for intervention versus placebo was reported to be 2.1 (95% confidence interval: 0.5-9.8). Liver enzyme effects were reported in both placebo and treatment groups and, where liver enzyme elevations were observed, effects were mild and no serious liver-related adverse events were reported. Results from this review, although not conclusive, suggest that liver-related adverse events following intake of green tea extracts are expected to be rare (57). The mechanisms of EGCG induced antioxidant activity and hepatotoxicity has been studied in animal models. (58). The mechanism of EGCG-triggered hepatotoxicity involves suppression of

major antioxidant enzymes and the Nrf2 rescue pathway plays a vital role in the counteraction of this EGCG toxicity. It is not clear whether upregulation of the Nrf2 pathway by EGCG contributes to the antioxidant property of EGCG in humans. The baseline and post treatment liver biopsies to be collected in our proposed clinical study will be used to study the mechanism of antioxidant effect of EGCG.

In some reviews, green tea extracts containing EGCG have been shown to improve liver function in participants with non-alcoholic fatty liver disease (NAFLD). (ref 59 and 60) The investigators concluded that upon ingestion of 700 ml per day of green tea containing > 1 g catechin, participants with NAFLD demonstrated improved liver fat content and reduced inflammation. Several mechanisms have been proposed to account for this liver protective effect of EGCG, including but not limited to reduction in liver oxidative stress, inhibitory activity against lipase, decreased mitochondrial β-oxidation, and non-heme iron absorption.

In this study, we are targeting participants with well-compensated cirrhosis (Child Pugh A) who have a high risk of developing HCC (annual incidence of 3-5%) and have no proven options for preventive treatment. A preventive therapy against HCC development is urgently needed. Given the rare liver toxicities and the effective HCC prevention observed in preclinical models, we believe that the evaluation of Polyphenon E, green tea extract, in subjects with a high risk of HCC development (high γ-OHPdG liver in cirrhotic liver) is worthwhile.

Cirrhotic participants are likely to have a baseline increase in transaminases. We deduce this from the fact that most participants are found to have cirrhosis only after having a blood test for an unrelated condition (such as chronic viral hepatitis, non-alcoholic steatohepatitis, or alcoholic liver disease) that causes elevated transaminases. A mild increase (</= 2.5 x upper normal level (UNL)) in transaminase does not reflect the level of liver function impairment in well-compensated cirrhosis. To ensure safety, subjects selected will have normal direct bilirubin, and synthetic function including albumin $\geq$ 3.0 g/dL and INR $\leq$ 1.3. Following FDA recommendations, weekly or bi-weekly (depending on assigned dose, clinical stability, and liver biochemical stability of the participant) liver function & coagulation tests will be carried out for safety monitoring. We propose to enroll participants with baseline AST/ALT that is less than 2.5 x UNL. Change of transaminase levels from baseline will be used to monitor the hepatotoxicity of Polyphenon E. In participants who develop changes in transaminases, we will hold treatment until levels recover to baseline OR discontinue therapy based on the extent of the increase of AST/ALT from the baseline level, according to the detailed dose modification/withholding drug scheme for elevated ALT/AST described in Section 5.6.

2.5.3 Rationale - Study Endpoints

Our preliminary data shows that γ-OHPdG predicts HCC development. Among the 3 anti-oxidants, including Theaphenon E, lipoic acid, and vitamin E, used in the 3 HCC animal models, only Theaphenon E effectively suppressed the accumulation of γ-OHPdG and prevented the HCC development. Theaphenon E contains the same active components—epigallocatechin gallate (EGCG), epigallocatechin (EGC), and epicatechin (EC)—as the dietary antioxidant, Polyphenon E whichhas been tested in many cancer prevention clinical trials. We propose that the accumulation of the DNA adduct, γ-OHPdG, predicts the development of HCC, and that Polyphenon E suppresses the formation of γ-OHPdG, which may reduce HCC development in participants with

cirrhosis. In this pilot study, our primary endpoint is to determine the effect of treatment on γ-OHPdG levels in cirrhotic liver. On enrollment, subjects will have a baseline biopsy taken. Only subjects found to have high γ-OHPdG levels in their cirrhotic liver will receive Polyphenon E treatment for 24 weeks. A repeat biopsy will be taken 6 months after treatment initiation, and will be compared with the baseline biopsy for changes in liver γ-OHPdG levels.

HCC animal model data support the ability of γ-OHPdG levels to predict the development of HCC. In addition, γ-OHPdG levels have been shown to correlate with GC to AT mutations, and other mutations in oncogene and tumor suppressor genes found in human HCC, which supports the mechanistic role of γ-OHPdG in hepatocarcinogenesis. In our preliminary study, we found that 38% of 68 cirrhotic liver samples expressed high level of γ-OHPdG. In order to test γ-OHPdG as a predictive biomarker for HCC development, we first need to collect additional data on the prevalence of high γ-OHPdG levels (IHC score of 3 or higher) in liver cirrhosis, the stability of γ-OHPdG levels during the course of cirrhosis, and the correlation of high γ-OHPdG levels (IHC score of 3 or higher) with the development of HCC. In this phase I study, secondary endpoints were chosen to estimate the fraction of participants with liver cirrhosis that have high levels of γ-OHPdG, and the stability of the γ-OHPdG marker over time in each individual. In addition, we will also test the effect of EGCG on the level of liver stiffness as measured by FibroScan® and FIB-4 score as an exploratory study endpoint. We will also develop an LC-MS and/or ELISA-based method for detecting urinary γ-OHPdG as an exploratory study endpoint. Lastly, it is unlikely that a patient will develop HCC during the short time they are on study. However, we will measure the incidence of HCC across all dosage levels, and have thus added it as an exploratory endpoint.

3. SUMMARY OF STUDY PLAN

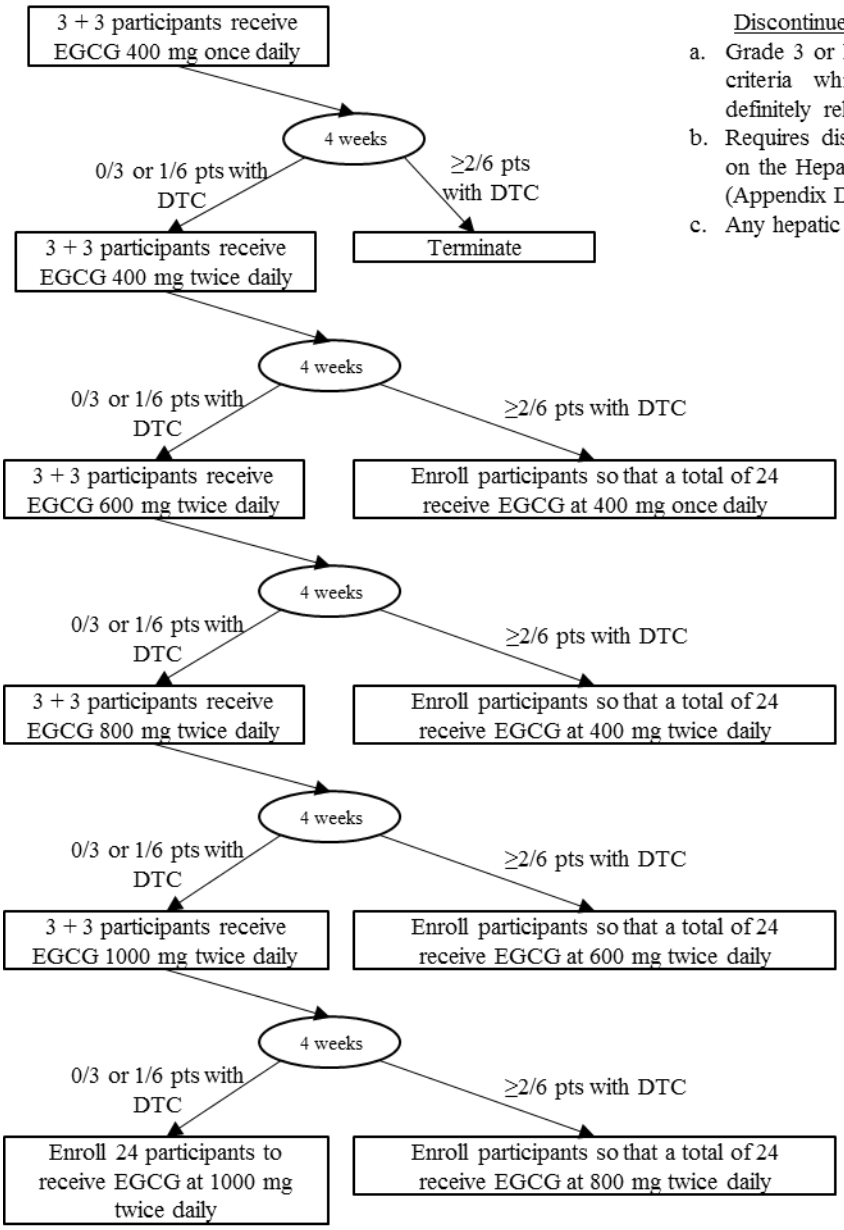
3.1 We propose a single arm, multicenter phase I study of subjects with cirrhotic livers expressing high levels (score 3 and above) of γ-OHPdG (γ-OHPdG-high) in their baseline biopsy (biopsy 1) who will receive Polyphenon E for 24 weeks. This study is a dose escalation design (see **Dose Escalation Schema** below). Participants in each cohort will be monitored for 4 weeks for Discontinue Therapy Criteria (DTC) before additional participants will be enrolled for the next dose cohort.

The Discontinue Therapy Criteria is defined as:

a. Grade 3 or higher toxicity based on CTCAE criteria which is possibly, probably, or definitely related to the study agent OR
b. Requires discontinuation of treatment based on the Hepatotoxicity Assessment Algorithm (Appendix D) OR
c. Any hepatic decompensation such as
    1. New onset ascites or worsening of ascites (requiring increase in drug therapy or requiring surgical intervention such as paracentesis).
    2. Variceal bleeding documented by endoscopy
    3. Spontaneous bacterial peritonitis documented by positive culture
    4. Hepatic encephalopathy
    5. Hepatorenal syndrome (Type 1 or 2)
    6. Porto-pulmonary hypertension

7. Hepato-pulmonary hypertension
8. Any liver related event that leads to hospitalization or G4 event.

Dose Escalation Shema



Discontinue Therapy Criteria (DTC):

a. Grade 3 or higher toxicity based on CTCAE criteria which is possibly, probably, or definitely related to the study agent OR
b. Requires discontinuation of treatment based on the Hepatotoxicity Assessment Algorithm (Appendix D) OR
c. Any hepatic decompensation such as
    1. New onset ascites or worsening of ascites (requiring increase in drug therapy or requiring surgical intervention such as paracentesis).
    2. Variceal bleeding documented by endoscopy
    3. Spontaneous bacterial peritonitis documented by positive culture
    4. Hepatic encephalopathy
    5. Hepatorenal syndrome (Type 1 or 2)
    6. Porto-pulmonary hypertension
    7. Hepato-pulmonary hypertension
    8. Any liver related event that leads to hospitalization or G4 event.

One month following the end of intervention (i.e. 7 months from study entry), subjects will have a post-therapy clinic visit, to be evaluated for the resolution of any side effect from the treatment and review of the six-month repeat scan results. All participants will have a repeat liver biopsy (exit biopsy) taken at the end of the 24 week intervention (see **Participant Flow Schema** at the beginning of this document). The Participant Flow Schema at the beginning of this document summarizes the sequence of events for participants. Details are not presented in the Schema, but can be found under Section 7.

In our unpublished data, 68 participants with cirrhosis, 38% (90% confidence interval, range 0.28, 0.49) of cirrhotic liver biopsies demonstrated high γ-OHPdG levels (IHC score ≥ 3). Therefore, a minimum of 4 and maximum of 171 participants with liver cirrhosis will be screened in our current study. A minimum of 2 and a maximum of 48 participants whose cirrhotic liver biopsies show a γ-OHPdG score ≥ 3 will be enrolled. If a maximum tolerated dose is established, a total of 24 participants will receive the maximum tolerated dose.

3.2 UNL

For the first six subjects who consent for additional PK collections at Georgetown University, blood will be collected for pharmacokinetic study on Visit 1 (day 1 of week 1): pre-dose and at 1.5, 3.5, and 8.5 hours (+/- 5 minutes) after the first dose of Polyphenon E under fed conditions. Study subjects will self-collect their urine before dosing and at three intervals during the 24-h period after dosing (0–4, 4–8, and 8–24 h)..

All subjects will have a clinic visit at Screen 1, Screen 2, Visit 1 (week 1 day 1), Visit 2 (week 13 +/- 3 days), Visit 3 (week 25 +/- 3 days, end of treatment), Visit 4 (week 28 +/- 7 days, end of study visit), described in detail in Section 7.1. The laboratory tests at Screen 1 and Visits 1-4 include CBC, chemistry and liver function tests (CMP), and coagulation tests. Following the FDA mandate, weekly or bi-weekly (depending on assigned dose, clinical stability, and liver biochemical stability of the participant) liver function & coagulation tests will be carried out for safety monitoring.

An ultrasound, CT or MRI scan will also be performed after consent and prior to starting agent unless it has been done per standard of care within three months from the consenting date and showed no evidence of HCC. It will also be performed at the end of intervention (Visit 3 $\pm$ 3 weeks) as part of standard of care. The imaging performed at the beginning and end of intervention must be the same method (i.e. if an ultrasound was used or performed during screening, then an ultrasound must be performed at the end of intervention). These evaluations will detect any HCC development during the treatment.

FibroScan will be performed after consent and prior to starting agent as part of standard of care unless the scan has been done within three months from the consenting date. It will also be performed at the end of intervention (Visit 3 $\pm$ 3 weeks) as a research procedure. These evaluations will evaluate the change of liver stiffness after six months of Polyphenon E treatment.

4. PARTICIPANT SELECTION

4.1 Inclusion Criteria

4.1.1 Participants with a clinical diagnosis of cirrhosis based on the investigators evaluation, confirmed by ANY ONE of the three following methods to define cirrhosis:

- Established cirrhosis on liver biopsy (METAVIR F4);
- Ultrasound, CT or MRI findings consistent with cirrhosis. Nodular appearing liver with or without evidence of portal hypertension

- Transient elastography (FibroScan) with a result > 12.5 kPa

Etiology of cirrhosis will not be considered in determining inclusion in the study.

4.1.2 Participant is able and willing to comply with study procedures, and signed and dated informed consent is obtained.

4.1.3 Participant agrees to consume no more than 2 cups of green tea per day and refrain from taking supplements or foods labeled as containing green tea.

4.1.4 Participant must be aged ≥ 18 years. Because no dosing or adverse event (AE) data are currently available on the use of Polyphenon E in participants < 18 years of age, children are excluded from this study but will be eligible for future pediatric trials, if applicable.

4.1.5 ECOG performance status ≤ 1 (Karnofsky ≥ 70%; see Appendix A).

4.1.6 Participants must have adequate organ and marrow function as defined below in Table 2:

Table 2:

System	Laboratory Value
Hematological	
Platelets	≥ 75,000 / μL
Hemoglobin	≥ 8 g/dL
Renal function	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	Within normal institutional limits **OR** GFR within normal institutional limits as adjusted for age and sex.
Hepatic function	
Serum direct bilirubin	Within normal institutional limits
AST (SGOT) and ALT (SGPT)	≤ 2.5 X UNL
Albumin	≥ 3.0 g/dL
Coagulation	
International Normalized Ratio (INR)	≤ 1.3
Other	
Ascites	Absent
Encephalopathy	Absent

4.1.7 Only participants found to express high levels (IHC score 3 and above) of γ-OHPdG (γ-OHPdG-high HCC) in baseline or archival liver biopsy will be registered to receive Polyphenon E treatment.

4.1.8 Participant is able to undergo radiographic evaluation with ultrasound, CT or MRI.

4.1.9 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). Contraception must be used 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. Female participants of childbearing potential should have a negative urine or serum pregnancy test within 72 hours prior to receiving the first dose of study medication (if a urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required).

4.2 Exclusion Criteria

4.2.1 Participant has confirmed HCC by ultrasound/CT/MRI. Participants who have previously had HCC but have been treated and have been recurrence free for 5 years are eligible.

4.2.2 Participant has or has had other cancer(s) within 3 years of study; however, in situ breast, in situ cervical, and basal cell/squamous cell skin cancers are allowed. Participant with active, other cancer that requires systemic therapy will be excluded from this study. Participant with early stage cancer that requires local therapy, such as cervical ablation for early stage cervical cancer, are allowed to be registered in the study and are allowed to receive local therapy.

4.2.3 Inability to swallow capsules.

4.2.4 Participant has a known diagnosis of mental incapacitation that may affect their ability to consent and be compliant with the protocol.

4.2.5 Participant has ever experienced one or more hepatic decompensation events or a history of decompensated liver disease as listed below:

- Clinical ascites
- Variceal bleeding documented by endoscopy
- Spontaneous bacterial peritonitis documented by positive culture
- Hepatic encephalopathy
- Hepatorenal syndrome (Type 1 or 2)
- Porto-pulmonary hypertension
- Hepato-pulmonary hypertension
- Any liver-related event which led to a hospitalization or a grade 4 event

4.2.6 Participant has an underlying predisposition to GI or rectal bleeding are considered ineligible for study participation.

4.2.7 History of allergic reactions attributed to compounds of similar chemical composition to Polyphenon E (or green tea). Note that participants who are unable to tolerate intravenous contrast for CT scans should have MRIs or ultrasounds during the study instead of CT scans.

4.2.8 Participant is receiving any other investigational agents.

4.2.9 Participants have taken supplements or foods that are labelled as containing green tea for 8 weeks before start of treatment.

4.2.10 Uncontrolled intercurrent illness 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. HBV and HCV infections are allowed.

4.2.11 Green tea has been consumed by humans for thousands of years and teratogenic or abortifacient effects have not been reported. However, subjects in this study will take high doses of Polyphenon E. The teratogenic or abortifacient effects of high dose Polyphenon E is unknown; therefore pregnant women are excluded from this study. Because there is an unknown but potential risk for AEs in nursing infants secondary to treatment of the mother with Polyphenon E, breastfeeding should be discontinued if the mother is treated with this study agent.

4.3 Inclusion of Women and Minorities

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

4.4 Recruitment and Retention Plan

The following strategies will be applied to ensure complete enrollment within the first twelve months of study initiation:

MedStar Georgetown Transplant Institute is a network of multidisciplinary teams that provides medical care to participants with chronic liver disease. This network includes GUH, Washington Hospital Center (WHC), Franklin Square Medical Center, and MedStar Georgetown Transplant Institutes in Fairfax and Frederick. The network serves about 2,200 participants with chronic liver disease each year. Dr. Coleman Smith has weekly clinics at GUH, Franklin Square Medical Center, and MedStar Georgetown Transplant Institutes in Fairfax. Dr. Rangnekar and Dr. Thomas have weekly clinics at the GUH, WHC and MedStar Georgetown Transplant Institute in Frederick. When they identify potential candidates at any of the sites (pre-screen), eligible and interested subjects will be scheduled for a Screen 1 visit at GUH in DC and enrolled if indeed eligible at GUH. GUH is the site with the largest patient volume, will provide 70-80% enrollment for this study under MedStar Georgetown transplant Institute. The distance between GUH to other sites ranges from 5 to 17 miles (travel time 15 min to 40 min). These distances allow participants relatively easy travel from other MedStar sites to GUH, although only about 20-30% of enrollment under MedStar Georgetown Transplant Institute (GUH) will be referred from other MedStar sites. The transplant institute at Frederick is 45 miles from Georgetown (approx 1 hr drive), but due to the small patient volume seen the transplant institute at Frederick, and not many participants are expected to be referred to GUH. Therefore, the distance between GUH and the transplant institute at Frederick will have minimal impact on enrollment.

Among the 2,200 hepatology participants seen under the MedStar Network within one year, approximately 20% have compensated cirrhosis without liver cancer. Therefore we have a pool of 440 participants that meet the eligibility criteria for this study. Based on our small 50-patient survey, 90% of participants should be interested in participating in the study, despite having to undergo liver biopsy. Thus we have a pool of 400 participants per year from which to draw potential study candidates.

At the University of Puerto Rico, over 1000 participants are seen annually over the hepatology clinical enterprise. It is estimated that approximately 50% of these participants have underlying cirrhosis at different stages, including pre-transplant participants. Dr. Cruz estimates that 10% of cirrhotic individuals may be eligible for the study translating to approximately 50-100 participants providing adequate numbers for screening into the study.

Monthly teleconferences will be arranged for Drs. Coleman Smith, Marcia Cruz, Victor Carlo, Rafael Pastrana, Ruth He, and the study coordinators to discuss enrollment and study related issues.

Our strategies are to prescreen most participants by medical record review after the protocol is approved, obtain consent, and, if needed, obtain a baseline biopsy from most participants within the first few months of study initiation so that candidates for intervention are identified quickly. From the pool of study candidates, we plan to screen 6 participants per month if possible. With this plan, even if there are delays for any reason, we will be able to complete the study within two years. We estimate 60% enrollment will occur at MedStar and 40% enrollment will occur at UPR.

We plan to allow up to three weeks from the day of biopsy to obtain the liver γ-OHPdG score. The liver samples will be batched and tested at least twice per month. If the rate of cirrhotic liver biopsies with a γ-OHPdG score ≥ 3 is lower than expected, we will increase the number of subjects that are pre-screened per month.

Participants will also be able to find trial details and sites through the Lombardi Cancer Center and Northwestern Cancer Prevention Consortium websites. In addition, the study investigators will work with advocacy groups across the country to improve awareness.

5. AGENT ADMINISTRATION

Polyphenon E will be administered on an outpatient basis. Reported AEs and potential risks are described in Section 6.2.

5.1 Dose Regimen and Dose Groups

The treatment to be used in this trial is outlined below in Table 3:

Table 3: Trial Treatment

Drug	Dose/ Potency	Dose Frequency	Route of Administration	Regimen/ Treatment Period	Use
Polyphenon E	400 mg EGCG	daily	oral	Continuously for 24 weeks	Experimental
	400mg EGCG	bid			
	600mg EGCG	bid			
	800mg EGCG	bid			
	1000mg EGCG	bid			

On an outpatient basis, Polyphenon E will be administered orally according the dose level of

EGCG assigned for each cohort on a continuous dosing schedule for 24 weeks.

The medication will be released on Visit 1 (week 1, day 1); and Visit 2 (week 13 +/-3 days). A participant diary will be provided to participants and collected from them by the study coordinator approximately every 12 weeks during clinic visits for the 24 weeks of treatment. Thus participants will have their diary reviewed and a pill count performed at their Visit 2 (week 13 +/- 3 days) and at Visit 3 (end of intervention, 25 +/- 3 days).

5.2 Polyphenon E Administration

The study agent will be self-administered by the subject. Participants will be instructed to take each dose of Polyphenon E after a light meal.

The medication will be released by the research pharmacies at GUH or UPR every 12 weeks; thus on Visit 1 (week 1, day 1), and Visit 2 (week 13 +/- 3 days). A participant diary will be provided. In order to give participants some flexibility for Visit 2 (week 13+/- 3 days), patient will be provided one extra week of medication, so they will not run out of medication before they receive the medication on Visit 2 (week 13 +/- 3 days). Participants will have their diary reviewed and a pill count performed at their Visit 2 (week 13 +/- 3 days) and Visit 3 (end of intervention, week 25 +/- 3 days).

Polyphenon E/EGCG will be administered orally at a doses of 400 mg qd, 400 mg bid, 600 mg bid, 800 mg bid, 1000 mg bid on a continuous dosing schedule for a total of 24 weeks, according to the dose escalation 3 + 3 design. Participants are instructed to take their medication after a light meal. The meal should not include either milk or juice during the meal. If a dose is missed, the participant should not make up the missing dose the next day, as this would result in an extra dose for that day. Participants should not make up vomited doses. The missing dose or vomited dose should be recorded in the medication diary.

Pharmacokinetics study:
The day before the pharmacokinetic study (Visit 1), study participants at Georgetown University are instructed to fast after midnight except for drinking water. On the pharmacokinetic study day, study subjects at Georgetown University skip breakfast and take no over the counter medications, vitamins, or health food products. Study participants at Georgetown University come to the clinic in the early morning (6–8 a.m.) and are provided with food for breakfast. Immediately after or during breakfast, study subjects swallow their Polyphenon E capsules with a glass of water. Study subjects are allowed unlimited water intake throughout the study day. Other drinks are not allowed. Blood samples (5–7 ml each) are collected before the administration of the Polyphenon E and then at time points 1.5, 3.5, and 8.5 hours (+/- 5 minutes) after drug administration. Study subjects self-collect urine before dosing and at each PK blood draw. After the 3.5 h blood collection, food for lunch is provided to the study subjects.

5.3 Run-in Procedures

Not applicable

5.4 Contraindications

Participants who consume 3 or more cups of green tea per day, or take vitamins or health food products that are labelled as containing green tea components.

5.5 Concomitant Medications

Medications specifically prohibited in the exclusion criteria are not allowed during the ongoing trial.

Acceptable Concomitant Medications
All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), vitamin and mineral supplements, herbal supplements, and IV medications and fluids. CRF reporting will include: 1) start and stop date, dose and route of administration, and indication. Medications taken for study procedures (e.g. biopsy) will not be included. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date should also be included on the CRF.

All concomitant medications received starting at Screen 1 or within 28 days of the first dose of trial treatment (whichever is earlier) and at every clinic visit should be recorded.

Prohibited Concomitant Medications
Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase (including retreatment for post-complete response relapse) of this trial:
- Investigational agents other than Polyphenon E
- Medications listed as prohibited in the trial Exclusion Criteria (Section 4.2).

Participants must be willing to refrain from drinking > 3 cups of green tea daily, or take supplements or foods that are labelled as containing green tea during the screening, intervention, and follow-up periods

There are no restrictions on other caffeine-containing beverages or over-the-counter medications.

5.6 Dose Interruption and Modification

Guidelines for Dose Interruption of Polyphenon E provided below are based on the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE Version 4.0) and the Hepatotoxicity Assessment Algorithm.

For any non-liver related toxicities, Polyphenon E can be continued for grade 1 adverse events attributed by an investigators to be possibly, probably, or definitely related to treatment.
Polyphenon E will be held for grade 2 adverse events attributed by an investigator to be possibly, probably, or definitely related to the treatment. In this case, Polyphenon E will be held until the adverse reaction resolves or improves to baseline, at which time Polyphenon E can be re-initiated at the same dose level. If the adverse reaction re-occurs at grade 2, Polyphenon E should be

permanently discontinued. Polyphenon E will be discontinued for grade 3 or 4 adverse events attributed by an investigator to be possibly, probably, or definitely related to the treatment.

Polyphenon E will be held for any elevation in liver function tests and investigators will determine the course of action according to Hepatotoxicity Assessment Algorithm (Appendix D) for details.

Hepatotoxicity Assessment Algorithm (**Appendix D**) is being used for hepatotoxicity safety monitoring, in conjunction with NCI CTCAE which is used for grading of adverse events. We will monitor participants' laboratory parameters every 1-2 weeks as recommended. Participants who do experience toxicity should be reassessed within 3-4 days of the toxicity for resolution. Regarding hepatotoxicity, the procedures for laboratory monitoring, treatment interruption, preinitiation, or permanent discontinuation are described in detail in Appendix D.

There is no plan for dose modification after patient has started Polyphenon E treatment.

5.7 Adherence/Compliance

5.7.1 All toxicity and samples from all subjects who receive drug will be evaluated and included in the primary analysis, which will be based on intent to treat principle.

5.7.2 The unused pill count and/or participant diary will be provided to and collected from participants by the study coordinator every 3 months during clinic visits for the 6 months of Polyphenon E treatment.

5.7.3 Additionally, an adequate record of receipt, distribution, and return of all study drugs must be kept in a pill count form for the pharmacist to complete.

5.7.4 Measuring the three major metabolites (EGCG, EGC, and EC) of green tea catechins in urine will provide additional evidence of compliance. Pill count and diary are usually used for compliance, however these parameters could be misreported or tampered by participants. For this purpose, urine samples will be collected at Visit 2 and Visit 3.

Subject compliance with the treatment and protocol includes willingness to comply with all aspects of the protocol, and to have blood collected for all safety evaluations. At the discretion of the principal investigator, a subject may be discontinued from the trial for non-compliance with follow-up visits or study drug.

6. PHARMACEUTICAL INFORMATION

6.1 Polyphenon E (IND [redacted], NCI/DCP)

Polyphenon E (Mitsui Norin Co., Ltd., Shizuoka, Japan) is a botanical drug substance containing a mixture of catechins originating from the leaves of green tea (*Camellia sinensis*). The active pharmaceutical ingredient is a purified tea fraction containing 80–98% total catechins by weight; the main component is epigallocatechin gallate (EGCG), which comprises 50–75% of the material.

Other catechins are present at levels of 12% or below each, including epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and gallocatechin gallate (GCG). Polyphenon E may also contain caffeine (<1.0%), theobromine (<1.0%), and gallic acid (<0.5%).

The investigational product to be used in the proposed clinical investigation is a Swedish orange, opaque, size 00 hard gelatin capsule containing enough Polyphenon E to deliver 200 mg EGCG/capsule. Inactive excipients in capsules include microcrystalline cellulose national formulary (NF), croscarmellose sodium NF, colloidal silicon dioxide NF, and magnesium stearate NF.

Clinical studies investigating the safety and chemopreventive efficacy of Polyphenon E are being conducted under NCI, DCP-sponsored IND [redacted], filed in FDA's Division of Oncology Products.

6.2 Reported Adverse Events and Potential Risks

Safety data are available from multiple NCI, DCP-sponsored clinical studies with Polyphenon E. In two completed single-dose phase 1 studies, a total of 50 subjects received Polyphenon E in dose levels from 200–1200 mg EGCG, in both fed and fasted conditions [64,65]. The most severe treatment-emergent adverse event (TEAE) was one grade 3 event of atrial fibrillation that was considered unlikely to be related to study medication. Frequently reported TEAEs included grade 1 and 2 headache, nausea, and abdominal pain, which in some cases were considered possibly or probably related to study drug. Reports of nausea and abdominal pain appeared to increase in frequency with increasing dose, especially under fasting conditions. Other TEAEs possibly or probably related to Polyphenon E administration did not have a clear dose-response relationship. Gastrointestinal (GI) AEs were usually mild, and seen most often in the fasting condition and at the highest dose level (1200 mg EGCG). Onset of GI events typically occurred within two to three 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.

Two NCI, DCP-sponsored multi-dose studies with Polyphenon E were conducted in healthy subjects, who were administered study drug at 400 mg EGCG twice daily (bid) (16 subjects) or 800 mg EGCG once daily (qd) (58 subjects) [64, 66]. All TEAEs were grade 1 or 2, with headache and nausea reported in >20% of subjects, abdominal pain in approximately 15% of subjects, and diarrhea, dizziness, and rhinitis in approximately 10% of subjects.

Five NCI, DCP-sponsored placebo-controlled multi-dose studies with Polyphenon E have been conducted, with dose levels ranging from 800–1200 mg EGCG qd to 200–800 mg EGCG bid; dose durations ranged from two weeks to six months [61-63]. Of the TEAEs that were reported, 98% were grade 1 or 2. The majority of subjects were given 800 mg EGCG qd (n=85), and the most commonly reported TEAEs at that dose were nausea (24% of subjects) and headache (18%). Headache and GI-related TEAEs, including diarrhea, nausea, and dyspepsia, were common across dose levels. These TEAEs tended to increase from 400 mg EGCG/day to 800 and 1200 mg EGCG/day; they were not reported at 1600 mg EGCG/day, perhaps due to the low number of subjects (n=3) at that dose level. These events were generally observed at a higher incidence in Polyphenon E-treated subjects compared with placebo subjects. Additional TEAEs that were

frequently reported included grade 1 laboratory abnormalities in a Barrett's esophagus study, with 23 subjects given 200, 400, or 600 mg EGCG bid.

In NCI, DCP-sponsored trials, as well as investigator-sponsored studies with Polyphenon E, grade 3 TEAEs considered probably or possibly related to study drug included abdominal pain, dyspepsia, diarrhea, fatigue, and rectal hemorrhage. The grade 3 rectal bleeding was unexpected; it is therefore recommended that participants with an underlying predisposition to GI or rectal bleeding be considered ineligible for study participation. These grade 3 TEAEs occurred in subjects administered 400–2000 mg EGCG bid. There were also three grade 3 incidences of increased alanine aminotransferase (ALT) considered probably or possibly related to study drug. These events occurred in a phase 2 chronic lymphocytic leukemia (CLL) study at 2000 mg EGCG bid [56], in a cervical cancer study at 800 mg EGCG qd [62], and in a breast cancer study at 800 mg EGCG bid [62].

The safety of tea and tea compounds is supported by centuries of human consumption. However, in recent years, oral use of green tea extracts (GTEs) has been associated with several instances of hepatotoxicity [67, 68]. Most affected participants were women, and many were consuming GTEs for the purpose of weight loss. 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 [redacted] 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. In addition, the sale of Exolise (an ethanolic GTE sold as a weight reduction aid) was suspended in Spain in 2003 after reports of hepatotoxicity (four cases in Spain and nine in France) associated with its use [69]. 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. These severe adverse reactions appear to be rare and have never been reported in any subject administered Polyphenon E. However, increased toxicity, including hepatotoxicity, is observed when Polyphenon E or EGCG is administered to fasted dogs. Therefore, the FDA Division of 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.

In nearly 30 completed multidose studies with Polyphenon E, including NCI, DCP- and investigator-sponsored, there have been >200 reports of elevated ALT or aspartate aminotransferase (AST), in approximately 5% of subjects. Of these events, 90% were grade 1, with 19 grade 2 events and four grade 3 events. Of the grade 2 events, nine occurred at 2000 mg EGCG bid, one at 1200 mg EGCG bid, three at 800 mg EGCG bid, four at 800 mg EGCG qd, and two at 400 mg EGCG bid. The grade 3 events occurred at 2000 mg EGCG bid, 1200 mg EGCG bid, 800 mg EGCG bid, and 800 mg EGCG qd. The majority of elevated ALT and AST events were reported in two open label Polyphenon E studies conducted in subjects with CLL [66, 70]. The phase 1 study evaluated the safety and preliminary efficacy of Polyphenon E at doses from 400 mg to 2000 mg EGCG bid for six months; subjects benefiting from treatment who had not

experienced unacceptable toxicity were offered up to one additional year of supplementation with either Polyphenon E or the GTE Polyphenon 100 (Mitsui Norin) at the same dose. Polyphenon 100 contains 120 mg GTE, and provides 100 mg catechins/capsule (65% EGCG). During this continuation portion of the trial, subjects had liver enzyme testing (ALT and AST) once each month. A phase 2 open-label study in the same cohort at 2000 mg EGCG bid for six months was also completed. The incidence of increased ALT and AST events (number of events/number of treated subjects) was approximately similar at doses of 400 and 800 mg EGCG bid (1.4–1.7 and 0.8–1.2 for ALT and AST, respectively); subjects at these dose levels were instructed to take the study medication on an empty stomach. At doses of 1000 mg EGCG bid and above, subjects were instructed to take the study medication within one hour of eating a substantial meal. The incidence of elevated ALT events at doses ≥1000 mg EGCG was 0.3–1.4 and 0.3–1.7 for ALT and AST, respectively. There was no obvious dose relationship at Polyphenon E doses of 1000−2000 mg EGCG bid taken with food. The incidence of events was generally higher at doses of 400 EGCG bid and 800 mg EGCG bid, taken on an empty stomach, compared with higher doses taken with food. Thus, there may be a decrease in frequency of ALT and AST elevations when study drug is administered on a full stomach; however, the subject numbers are small.

6.3 Availability

Polyphenon E is investigational agents supplied to investigators by NCI, DCP.
Table 5 describes supplies to be provided by DCP, NCI.

Table 5

Product Name & Potency	Dosage Form
Polyphenon E	Capsule (200 mg EGCG)

6.4 Agent Distribution

Agents will only be released by NCI, DCP after documentation of IRB approval of the DCP-approved protocol and consent is provided to DCP and the collection of all Essential Documents is complete (see DCP website for description of Essential Documents).

NCI, DCP-supplied agents may be requested by the Investigator (or their authorized designees) at each Organization. DCP guidelines require that the agent be shipped directly to the institution or site where the agent will be prepared and administered. DCP does not permit the transfer of agents between institutions (unless prior approval from DCP is obtained). DCP does not automatically ship agents; the site must make a request. Agents are requested by completing the DCP Clinical Drug Request form (NIH-986) (to include complete shipping contact information) and faxing or mailing the form to the DCP agent repository contractor:

John Cookinham
MRIGlobal
DCP Repository
1222 Ozark Street
North Kansas City, MO 64116

Phone: (816) 360-3805
FAX: (816) 753-5359
Emergency Telephone: (816) 360-3800

6.5 Agent Accountability

The Investigator, or a responsible party designated by the Investigator, must maintain a careful record of the inventory and disposition of all agents received from DCP using the NCI Drug Accountability Record Form (DARF). The Investigator is required to maintain adequate records of receipt, dispensing and final disposition of study agent. This responsibility has been delegated to GUH research pharmacy, UPR research pharmacy, and the principal investigator. The receipt record must include from whom the agent was received and to who study agent was shipped, as well as the date, quantity and batch or lot number. The dispensing record must note quantities and dates that the study agent was dispensed to and returned by each participant.

6.6 Packaging and Labeling

Polphenon E will be labeled and packaged by NCI, DCP.

Clinical supplies (Polyphenon E) will be affixed with a clinical label in accordance with regulatory requirements.

6.7 Storage

Study drug will be stored in a secure room temperature location, between 15–30°C.

An authorized person at the trial site must record receipt and dispensing of trial medication. Clinical supplies may not be used for any purpose other than that stated in the protocol.

6.8 Registration/Randomization

1. Laboratory tests for screening or study entry should be performed within 60 days prior to registration.
2. A study coordinator must upload into the Northwestern Clinical Trials Management System, a signed and complete informed consent and a completed eligibility form for each participant identified as eligible to be entered into the study.
3. All participants must be registered in the Northwestern University Robert H. Lurie Comprehensive Cancer Center Clinical Trials Management System (CTMS). Participants must not start protocol treatment prior to registration approval by the NCPC.
4. An eligibility checklist must be signed by a physician and uploaded into the CTMS. The pathology source documentation will be submitted and reviewed by NCPC quality checkers prior to registration approval (evidence of cirrhosis and y-OHPdG score).
5. When possible, the study coordinator will notify an NCPC Quality Assurance Monitor and/or send an email to ncpc@northwestern.edu prior to registering a participant. Prior notification is required for participant randomizations outside the normal business hours of Monday-Friday 9:00am-5:00pm CT.

6. After registration approval, participants will be assigned a participant identification number.
7. The clinical research coordinator and the research pharmacist will receive a participant identification number code for the patient via email.

6.9 Blinding and Unblinding Methods

This trial is a single-arm, open-label study.

DCP Medical/Task Order Monitor
Luz Maria Rodriguez, MD, FACS
NCI/Division of Cancer Prevention
9609 Medical Center Dr.
Rm 5E-228
Bethesda, MD 20892
Telephone (240) 276-7039
Fax (240) 276-7848
Email: rodrigul@mail.nih.gov

6.10 Agent Destruction/Disposal

The investigator is responsible for keeping accurate records of the clinical supplies received from DCP, NCI, the amount dispensed to and returned by the subjects, and the amount remaining at the conclusion of the trial.

Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed according to local institutional procedures. Destruction records must be provided to MRIGlobal at the end of the study.

7. CLINICAL EVALUATIONS AND PROCEDURES

7.1 Schedule of Events

	Pre-Screen	Screen 1[a] (0-60 days prior to registration)	Screen 2[a] (0-60 days prior to registration)	Visit 1: Week1 Day 1 (0-14 days after registration)	Between Visits 1 and Visit 2	Visit 2: Week 13 +/- 3 days)	Between Visits 2 and 3	Visit 3: End of Treatment (week 25 +/- 3 days)	Visit 4: End of Study (week 28+/- 7 days)
Pre Screen Eligibility performed by investigator[b]	x								
Informed consent		x							
Medical History		x							
Concomitant Medications Collection		x		x		x		x	
Adverse Events Assessments				x		x		x	
Physical Examination[c]		x		x		x		x	x
Vital Signs (temperature, pulse, respiratory rate, and blood pressure)[c]		x		x		x		x	x
Pregnancy test		x		x[d]					
Ultrasound, CT or MRI Scan Abdomen[e]		x						x	
FibroScan[f]		x						x	
Venipuncture for clinical labs		x		x		x		x	x
Hematology (CBC w/ diff and platelets)		x		x		x		x	x
Coagulation: INR		x		x	x	x	x	x	x
Comprehensive Metabolic Panel (CMP)[g]		x		x		x		x	x
Liver function tests[h]		x		x	x	x	x	x	x
Child Pugh Score[i]		x						x	
AFP (alpha-fetoprotein) and AFP-L3		x		x		x		x	x
Biopsy			X[j]					x	
Pathologist Review	x		x					x	
Research Urine collection[k]			x			x		x	
Research Blood collection[l]				x		x		x	
Research Blood PKs[m]				x					
Research Urine PKs[m]				x					
Alcohol and Tobacco Use Assessments[n]				x				x	
Dispensation of study drug[o]				x		x			

a. All subjects will be consented for study, have clinical visit, physical examination/vital signs, and laboratory test. Screen 1 and Screen 2 visit procedures may be combined or split into multiple visits,

as long as they occur within the window specified. Participants should not have liver biopsy until after confirmation of Screen 1 clinical blood lab results.

b. All subjects will first receive pre-screen for the diagnosis of cirrhosis and eligibility criteria by medical chart review.
c. All subjects will have a clinic visit and laboratory test at Screen 1, Visit 1 (week 1 day 1), Visit 2 (week 13 +/- 3 days), Visit 3 (week 25 +/- 3 days, end of treatment), Visit 4 (week 28 +/- 7 days, end of study visit). The laboratory tests at each visit include CBC with differential and platelets, CMP including BUN, creatinine, sodium, potassium, chloride, calcium, bicarbonate, glucose, chemistry and liver function tests including AST, ALT, alkaline phosphatase, total protein, albumin, direct bilirubin, indirect bilirubin, total bilirubin, magnesium, GGT, and phosphorus, coagulation tests INR, and AFP, AFP-L3.
d. If the participant is pre-menopausal and of child-bearing potential, and has not had a negative pregnancy test within 72 hours prior to starting study agent, another pregnancy test will be performed at this visit. If a test is performed, the result must be negative in order to start study agent.
e. All subjects will have undergone imaging (ultrasound, CT or MRI) to exclude HCC, performed as part of standard of care after consent and before starting agent unless the imaging has been done within three months from the consenting date. Imaging will be performed at the end of the intervention period (Visit 3 $\pm$ 3 weeks) as part of standard of care. When one type of scan is selected, the scans chosen thereafter should be the same type during the period of time that the participant is treated or followed on the study.
f. Fibroscan is performed as part of standard of care after consent and before starting agent unless it has been done within three months from the consenting date. It is performed at the end of intervention (Visit 3 $\pm$ 3 weeks) as a research procedure.
g. The CMP includes BUN, creatinine, sodium, potassium, chloride, calcium, bicarbonate, and glucose.
h. In between Visits 1 and 2, and between Visits 2 and 3, liver function & coagulation tests including AST, ALT, alkaline phosphatase, total protein, albumin, direct bilirubin, indirect bilirubin, total bilirubin, magnesium, GGT, calcium, phosphorus, and INR will be performed according to the schedule below. These tests will be performed at the central lab, or, if the participant prefers, at a local clinic lab. Participants who experience hepatotoxicity according to Appendix D will be re-evaluated every 3-4 days until resolution.
    a. For participants assigned to 400 mg EGCG QD or 400 mg EGCG BID: bi-weekly liver function & coagulation testing (every 14 +/- 3 days).
    b. For participant assigned to ≥600 mg EGCG BID: weekly liver function & coagulation testing (every 7 +/-3 days) for the first 2 weeks on agent, or until the participant demonstrates stability of clinical condition and liver biochemical enzymes* for at least 2 weeks, whichever is longer. Thereafter, the participant will receive bi-weekly liver function & coagulation testing (every 14 +/- 3 days).
    *Stability of clinical condition and liver biochemical enzymes is defined as meeting all of the following, as listed in Appendix D:
        a. No symptoms of clinical hepatitis (vomiting, nausea, right upper quadrant pain)
        b. No ALT or AST increases to ≥5x upper limit of normal
        c. No ALT or AST increases to ≥3x baseline levels
        d. No elevations in direct bilirubin > 1.5 ULN
        e. No elevation of direct bilirubin > ULN, regardless of ALT or AST levels, with indicators of immunological reaction (i.e., rash or >5% eosinophilia).
i. For details on the Child-Pugh score, see https://www.qxmd.com/calculate/calculator_43/child-pugh-score.
j. Only the subjects with γ-OHPdG-high in the base-line liver biopsy will be registered for treatment. The treatment is to start within 14 days from registration. The subjects with γ-OHPdG-low liver

will have one follow up visit to discuss the test result and surveillance plan. Liver biopsy is required for participants who have no archival liver biopsy tissue adequate for γ-OHPdG testing collected and stored within 12 months prior to consent. Participants with archival liver biopsy tissue adequate for γ-OHPdG testing, and collected and stored within 12 months prior to consent, may optionally have the liver biopsy at Screen 2. If the participant has archival liver biopsy tissue collected within the last 12 months prior to consent, the archival tissue can be tested (or sent for testing) for γ-OHPdG at either Screen 1 or Screen 2.

k. Urine samples for compliance will be collected at Visit 2 and Visit 3. Urine sample for biomarker analysis will be collected at Screen 2 or Visit 1 if participant has archival biopsy.
l. Two green and two purple top tubes are collected. At Visit 1, the Research Blood should be collected prior to agent administration.
m. First six participantsparticipants who consented for PK sample collection at Georgetown: Participants will be asked to fast after midnight except for drinking water prior to coming into the clinic. Blood and Urine samples for pharmacokinetic analysis will be obtained on Visit 1 (week 1 day 1) in clinic prior to Polyphenon E treatment, and at 1.5 hour, 3.5 hour, and 8.5 hours (+/-5 minutes for blood PKs, and +/- 15 minutes for urine collection) for participantspartcipants receiving Polyphenon E treatment after a light breakfast. Urine samples are self-collected as indicated in section 10.2.
n. The Alcohol and Tobacco Assessments are found in Appendices E-H. The Baseline questionnaires (Appendices E & F) should be administered at Visit 1. The Follow-up Questionnaires (Appendices G & H) should be administered at Visit 3 or at the end of study treatment. Appendix I should be given to each participant upon completing the Alcohol and Tobacco Use Assessment the first time.
o. Polyphenon E will be dispensed at Visit 1 (week 1 day 1) (3 month supply) and Visit 2 (week 13 +/- 3 days) (3 month supply). Participant diary will be collected when the drug is dispensed. Unused pills will be counted at Visit 2 (week 13 +/- 3 days) visit and Visit 3 (week 25 +/- 3 days).

7.2 Baseline Testing/Prestudy Evaluation

Pre-Screening Evaluation
The medical charts for the proposed study eligibility (pre-screen) will be reviewed.

Screen 1 Visit
Participants who wish to be considered for registration will undergo a screening evaluation. A complete history and physical exam will be obtained at the screening visit. Additionally, labs will be ordered/reviewed to assess eligibility, and imaging will be reviewed to assess cirrhosis.

- Informed Consent
- Medical History
- Record Concomitant Medications
- Physical Exam & Vital Signs
- Ultrasound, CT or MRI of the abdomen will be performed at Screen 1, Screen 2, or at Visit 1 prior to starting agent, if not performed within 3 months prior to consent
- FibroScan will be performed at Screen 1, Screen 2, or at Visit 1 prior to starting agent, if not performed within 3 months prior to consent
- Clinical Labs – including CBC with differential and platelets, comprehensive metabolic panel including BUN, creatinine, sodium, potassium, chloride, calcium, bicarbonate, glucose, liver function tests including AST, ALT alkaline phosphatase, total protein, albumin, direct bilirubin, indirect bilirubin, and total bilirubin, magnesium, GGT, and phosphorous, coagulation test (INR), AFP and AFP-L3.

- Calculate Child Pugh score
- Pregnancy Test (only offered for premenopausal women with child-bearing potential).
- If the participant has archival liver biopsy tissue collected within the last 12 months prior to consent, the archival tissue can be tested (or sent for testing) for γ-OHPdG at either Screen 1 or Screen 2.

Screen 1 Visit will be performed 0-60 days prior to registration. Screen 1 and Screen 2 visit procedures may be combined or split into multiple visits, as long as they occur within the window specified. Participants should not have liver biopsy until after confirmation of Screen 1 clinical blood lab results.

Screen 2 Visit

Participants who meet the Screen 1 eligibility criteria, will return for the Screen 2 Visit.

- Ultrasound, CT or MRI of the abdomen will be performed at Screen 1, Screen 2, or at Visit 1 prior to starting agent, if not performed within 3 months prior to consent
- FibroScan will be performed at Screen 1, Screen 2, or at Visit 1 prior to starting agent, if not performed within 3 months prior to consent
- Liver biopsy is required for participants who have no archival liver biopsy tissue adequate for γ-OHPdG testing collected and stored within 12 months prior to consent. Participants with archival liver biopsy tissue adequate for γ-OHPdG testing and collected and stored within 12 months prior to consent, may optionally have the liver biopsy at Screen 2.
- If the participant has archival liver biopsy tissue collected within 12 months prior to consent, the archival tissue can be tested (or sent for testing) for γ-OHPdG at either Screen 1 or Screen 2.
- Research Urine for γ-OHPdG level
- The liver biopsy samples will be tested for γ-OHPdG level, if this was not done previously
- The pathology slides will be reviewed and scored for the γ-OHPdG level by pathologists.

Screen 2 Visit will be performed 0-60 days prior to registration. Screen 1 and Screen 2 visit procedures may be combined or split into multiple visits, as long as they occur within the window specified. Participants should not have liver biopsy until after confirmation of Screen 1 clinical blood lab results.

Registration and Randomization

Participants will be registered between Screen 2 and Visit 1 (week 1 day 1). For more information, see protocol section 6.8 Registration. There is no randomization in this study.

7.3 Evaluation During Study Intervention

Study Visit 1 (Week 1 Day 1)

Participants will come to the clinic to receive study procedures and their first dose of study agent. Participants who volunteer for the PK tests at Georgetown will be asked to fast after midnight except for drinking water prior to coming into the clinic.

- Ultrasound, CT or MRI of the abdomen will be performed at Screen 1, Screen 2, or at Visit 1 prior to starting agent, if not performed within 3 months prior to consent
- FibroScan will be performed at Screen 1, Screen 2, or at Visit 1 prior to starting agent, if

not performed within 3 months prior to consent
- If the participant is pre-menopausal and of child-bearing potential, and has not had a negative pregnancy test within 72 hours prior to starting study agent, another pregnancy test will be performed at this visit. If a test is performed, the result must be negative in order to start agent.
- Record Concomitant Medications
- Physical Exam & Vital Signs
- Adverse Events Assessments.
- Venipuncture for clinical labs
- Clinical Labs – including CBC with differential and platelets, comprehensive metabolic panel including BUN, creatinine, sodium, potassium, chloride, calcium, bicarbonate, glucose, liver function tests including AST, ALT alkaline phosphatase, total protein, albumin, direct bilirubin, indirect bilirubin, and total bilirubin, magnesium, GGT, and phosphorous, coagulation test (INR), AFP and AFP-L3.
- Administer the Alcohol and Tobacco Baseline Assessments (Appendices E & F)
- Provide participant with the participant diary (Appendix C), agent study drug instructions (Appendix B), and the resources handout (Appendix I).
- Research Blood (for γ-OHPdG level)
- Research Blood PKs (for the first six participants at Georgetown University who agree)
- Research Urine PKs (for the first six participants at Georgetown University who agree)
- Research Urine for γ-OHPdG level if participant had archival biopsy
- Dispensation of study drug

Study Visit 1 will be performed 0-14 days after registration.

Between Visits 1 and 2

Participants will complete weekly or bi-weekly liver function & coagulation tests according to the chart below. These tests can be done with local lab or MD, for convenience; LabCorp or Quest are preferred, as the results will be reported directly to hospital electronic medical chart. If another lab is used, the report can be requested to be faxed to the investigator and/or study coordinator.

- Venipuncture for clinical labs
- Liver function tests, including ALT, AST, direct bilirubin, indirect bilirubin, and total bilirubin, alkaline phosphatase, total protein, albumin, GGT, calcium, phosphorous, and magnesium
- Coagulation test (INR)

Assigned Dose	**Schedule of liver function & coagulation tests**
400 mg EGCG QD or 400 mg EGCG BID	Bi-weekly liver function & coagulation testing (every 14 +/- 3 days)
≥600 mg EGCG BID	Weekly liver function & coagulation testing (every 7 +/-3 days) for the first 2 weeks on agent, or until the participant demonstrates stability of clinical condition and liver biochemical enzymes* for at least 2 weeks, whichever is longer. Thereafter, the participant will receive bi-weekly liver function & coagulation testing (every 14 +/- 3 days).
* Stability of clinical condition and liver biochemical enzymes is defined as meeting all of the following, as listed in Appendix D:	

a. No symptoms of clinical hepatitis (vomiting, nausea, right upper quadrant pain)
b. No ALT or AST increases to ≥5x upper limit of normal
c. No ALT or AST increases to ≥3x baseline levels
d. No elevations in direct bilirubin > 1.5 x ULN.
e. No elevation of direct bilirubin > ULN, regardless of ALT or AST levels, with indicators of immunological reaction (i.e., rash or >5% eosinophilia).

Study Visit 2 (Week 13 +/-3 days)
After taking the study medication for 12 weeks +/-3 days, participants will return to the clinic.

- Record Concomitant Medications
- Physical Exam & Vital Signs
- Adverse Events Assessments.
- Venipuncture for clinical labs
- Clinical Labs – including CBC with differential and platelets, comprehensive metabolic panel including BUN, creatinine, sodium, potassium, chloride, calcium, bicarbonate, glucose, liver function tests including AST, ALT alkaline phosphatase, total protein, albumin, direct bilirubin, indirect bilirubin, total bilirubin, magnesium, GGT, and phosphorous, coagulation test (INR), AFP and AFP-L3.
- Research Urine collection (compliance)
- Research Blood collection (for γ-OHPdG level)
- Dispensation of study drug
- Participant diary review and unused pill count
- Give participant blank participant diary

Between Visits 2 and 3
Participants will complete weekly or bi-weekly liver function & coagulation tests according to the chart below. These tests can be done with local lab or MD, for convenience; LabCorp or Quest are preferred, as the results will be reported directly to hospital electronic medical chart. If another lab is used, the report can be requested to be faxed to the investigator and/or study coordinator.

- Venipuncture for clinical labs
- Liver function tests, including ALT, AST, direct bilirubin, indirect bilirubin, total bilirubin, alkaline phosphatase, total protein, albumin, GGT, calcium, phosphorous, and magnesium
- Coagulation test (INR)

Assigned Dose	**Schedule of liver function & coagulation tests**
400 mg EGCG QD or 400 mg EGCG BID	Bi-weekly liver function & coagulation testing (every 14 +/- 3 days)
≥600 mg EGCG BID	If the participant has previously demonstrated stability of clinical condition and liver biochemical enzymes* for at least 2 weeks, the participant will receive bi-weekly liver function & coagulation testing (every 14 +/- 3 days). If the participant has not already demonstrated stability of clinical condition and liver biochemical enzymes* for at least 2 weeks, the participant will receive weekly liver function & coagulation testing

	(every 7 +/-3 days) until the participant demonstrates stability* for at least 2 weeks. Thereafter, the participant will receive bi-weekly liver function & coagulation testing (every 14 +/- 3 days).
* Stability of clinical condition and liver biochemical enzymes is defined as meeting all of the following, as listed in Appendix D: a. No symptoms of clinical hepatitis (vomiting, nausea, right upper quadrant pain) b. No ALT or AST increases to ≥5x upper limit of normal c. No ALT or AST increases to ≥3x baseline levels d. No elevations in direct bilirubin > 1.5 x ULN. e. No elevation of direct bilirubin > ULN, regardless of ALT or AST levels, with indicators of immunological reaction (i.e., rash or >5% eosinophilia).	

7.4 Evaluation at Completion of Study Intervention

Study Visit 3 (Week 25 +/- 3 days)
After their last day of taking the study agent, the participant will come in for a clinic visit.

- Record Concomitant Medications
- Physical Exam & Vital Signs
- Adverse Events Assessments.
- Administer the Alcohol and Tobacco Follow-up Questionnaire (Appendices G & H)
- Venipuncture for clinical labs
- Clinical Labs – including CBC with differential and platelets, comprehensive metabolic panel including BUN, creatinine, sodium, potassium, chloride, calcium, bicarbonate, glucose, liver function tests including AST, ALT alkaline phosphatase, total protein, albumin, direct bilirubin, indirect bilirubin, total bilirubin, magnesium, GGT, and phosphorous, coagulation test (INR), AFP and AFP-L3.
- Research Urine Collection (compliance)
- Research Blood collection (for γ-OHPdG level)
- Calculate Child Pugh score
- Participant diary review and unused pill count
- Ultrasound, CT or MRI of the abdomen (this procedure can be performed at a separate visit ± 3 weeks from the Visit 3 date). The same imaging performed during screening should be performed at Study Visit 3.
- FibroScan (this procedure can be performed at a separate visit ± 3 weeks from the Visit 3 date).
- Liver biopsy

7.5 Post-intervention Follow-up Period/ Final Evaluations, Off Study

Study Visit 4 (Week 28 +/- 3 days)
After a 3 week follow-up, participants will return to the clinic.

- Physical Exam & Vital Signs
- Venipuncture for clinical labs
- Clinical Labs – including CBC with differential and platelets, comprehensive metabolic panel including BUN, creatinine, sodium, potassium, chloride, calcium, bicarbonate, glucose, liver function tests including AST, ALT alkaline phosphatase, total protein,

albumin, direct bilirubin, indirect bilirubin, and total bilirubin, magnesium, GGT, and phosphorous, coagulation test (INR), AFP and AFP-L3.

7.6 Methods for Clinical Procedures

Informed Consent
The Investigator must obtain documented consent from each potential subject prior to participating in this clinical trial. Consent must be documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion. A copy of the signed and dated consent form should be given to the subject before participation in the trial. The initial informed consent form, any subsequent revised written informed consent form, and any written information provided to the subject must receive the IRB/ERC's approval/favorable opinion in advance of use. The subject or his/her legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the subject's dated signature or by the subject's legally acceptable representative's dated signature. Specifics about the trial and trial population will be added to the consent form template at the protocol level. The informed consent will adhere to IRB/ERC requirements, applicable laws and regulations, and Sponsor requirements.

Inclusion/Exclusion Criteria
All inclusion and exclusion criteria will be reviewed by a study physician to ensure that the subject qualifies for the trial. See protocol section 6.8 Registration/Randomization for more information.

Medical History and physical exam (H&P)
A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the Investigator.
Information collected in the H&P is as follows:

1. All prescription, herbal medications, vitamins, and supplements taken, starting at Screen 1 or 28 days prior to starting study agent, whichever is earlier. Any medication or vaccine (including over-the-counter or prescription medicines, vitamins and/or herbal supplements) that the subject is receiving at Screen 1 up to the Final Visit must be recorded in source documents and the case report forms (CRFs). The reason for use, date(s) of administration (including start and end dates), and dosage information (including dose and frequency) must be recorded. Any change in concomitant therapy during the study period must be similarly recorded. Questions regarding prior or concomitant therapy should be directed to one of the investigators.
2. Vital signs including history of weight change.
3. Complete physical exam.
4. Laboratory study results must be obtained within 60 days prior to registration include the following:

- Complete blood count (CBC) with differential and platelets.

- Serum chemistries:
    - Complete Metabolic Panel including BUN, creatinine, sodium, potassium, chloride, calcium, bicarbonate, glucose
    - Liver function tests including AST, ALT, alkaline phosphatase, total protein, albumin, direct bilirubin, indirect bilirubin, total bilirubin, magnesium, GGT, calcium, and phosphorus.
    - Coagulation test (INR)
    - Tumor marker- AFP, AFP-L3.
- For women of child bearing potential, a serum β-HCG pregnancy test. This is for premenopausal women are those who have had the last menstrual period (LMP) within the last 12 months OR those who have had a hysterectomy, but have at least one intact ovary and are under age 55. Premenopausal women with child-bearing potential are those with at least one ovary, an intact uterus, and LMP less than 12 months previously
- Radiographic evaluation of cirrhosis or development of HCC with FibroScan and either ultrasound, CT or MRI.

Prior Medications Review
The investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the subject starting at Screen 1 or within 28 days before starting study agent, whichever is earlier. Treatment for the disease for which the subject has registered in this study will be recorded separately and not listed as a prior medication.

Concomitant Medications Review
The investigator or qualified designee will record medication, if any, that will be taken by the subject during the trial. All medications related to reportable SAEs and ECIs should be recorded as defined.

Disease Details and Treatments
The investigator or qualified designee will obtain prior and current details regarding disease status, and review all prior treatments including those for HBV and HCV.

Assignment of Screening Number
All participant records and tissue specimens will be de-identified using a letter and number assigned to their case at the time of screening. Screening ID blocks will be provided by Northwestern, and assigned to the participant by the accrual site at the time of screening. No record or specimen will contain information that could identify the participant. The key that connects participant identifiable information with this assigned number will be held by the Principal investigator. For computer records, the key will be protected by a double password protection system. Any paper records will be contained in a locked cabinet within a locked office to ensure participants' privacy is protected.

Biopsies
Of note, participants who are on chronic anticoagulation will be required to hold anticoagulation prior to the biopsies being performed. Participants on warfarin must hold treatment for 5 days, but will be on low-molecular weight heparin (LMWH), 1 mg/kg subcutaneously twice a day. LMWH

will continue until the evening prior to the biopsy. LMWH will be held in the morning of the procedure, but the patient will receive LMWH the evening of the day of biopsy. Participant may then resume warfarin the day of biopsy.

Preferred biopsy is core biopsy; 14 -18 gauge (G), 2.11-1.27 mm outer diameter (OD), 1.6-0.84 mm inner diameter (ID), 0.254-0.216 mm wall thickness, hypodermic needles can be used to perform the core biopsies based on the sites standard of care procedures. The use of a 16G needle is highly recommended as it is the most appropriate size to use for percutaneous liver biopsies (70,71). Twenty (20) unstained liver biopsy sections, cut 4-microns-thick must be submitted to the Dr. Chung's laboratory at Georgetown University/Lombardi for assessment of γ-OHPdG levels in liver samples. Subjects with high levels of γ-OHPdG (IHC stain =/> 3) who meet all other eligibility criteria will then be receive Polyphenon E treatment. A random liver biopsy will be performed at 24 weeks (+/- 7 days) prior to end of treatment. The liver tissue will be evaluated by Dr. Chung's laboratory at Georgetown University/Lombardi to assess for γ-OHPdG levels (Section 10.2). Liver biopsy samples will be stored in the lab of Dr. Fung-Lung Chung. γ-OHPdG testing of the liver biopsy will be carried out at least twice each month at Dr. Chung's lab. The liver biopsy samples will be shipped Monday-Thursday, after communication with the receiving site.

Lastly, all liver biopsies taken during the course of the study, regardless of the indication (i.e., both protocol-defined and those performed for other clinical indications) must be assessed by the PIs and the pathologists at Georgetown University Hospital.

Research Blood (for γ-OHPdG level)
At visits 1, 2, and 3, two green top tubes of blood will be drawn. The Research Blood at Visit 1 should be collected prior to agent administration. These samples will be uses exclusively for developing non-invasive methods for testing biomarker detection.

Research Urine (for γ-OHPdG level and compliance)
At Screen 2 and visits 2 and 3, one 100mL Urine sample will be collected. The Research Urine at Screen 2 will be used for γ-OHPdG levels and at visit 2 and 3 will be used for compliance to measure catechin levels.

Research Blood and Urine PKs
Six participants at Georgetown University will be given the option of participating in extra Research Blood and Urine PKs at Visit 1. The day before the pharmacokinetic study (Visit 1), Georgetown University study participants are instructed to fast after midnight except for drinking water. On the pharmacokinetic study day, study subjects skip breakfast and take no over the counter medications, vitamins, or health food products. Study participants come to the clinic in the early morning (6–8 a.m.) and are provided with food for breakfast. Immediately after or during breakfast, study subjects swallow their Polyphenon E capsules with a glass of water. Georgetown University study subjects are allowed unlimited water intake throughout the study day. Other drinks are not allowed. Two green top tubes of blood are collected before the administration of the Polyphenon E and then at time points 1.5, 3.5, and 8.5 hours (+/- 5 minutes) after drug administration. Study subjects self-collect urine before dosing and at each PK blood draw. After the 3.5 h blood collection, food for lunch is provided to the study subjects.

Study Agent
Participants will receive Polyphenon E on Visit 1 (week 1 day 1) and Visit 2 (week 13 ± 3 days) for a twelve week supply of 840 pills. To give some flexibility to participants for Visit 2 (week 13), on Visit 1 (week 1 day 1), participants will be given one extra week drug supply of additional 70 pills, therefore on Visit 1 (week 1 day 1) participants will be given a supply of study agent of 910 pills, while on Visit 2 (week 13+/- 3 days), participant will be given a supply of study agent of 840 pills.

Adverse Event (AE) Monitoring
The investigator or qualified designee will assess each subject to evaluate potential new or worsening AEs as specified in 7.1 SCHEDULE OF EVENTS, and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 4.0 (see Section 11.2). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

Full Physical Exam
The investigator or qualified designee will perform a complete physical exam and vital signs assessment during the screening period. Clinically significant abnormal findings should be recorded as medical history.

Vital Signs
The investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment and at treatment discontinuation as specified in the protocol section 7.1 Schedule of Events. Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only.

Liver and Abdominal MRI/CT scans, and Other Imaging
A comprehensive liver and abdominal ultrasound, MRI or CT scan will be performed at the Screen 1 or Screen 2 Visit if not performed within 3 months prior to consent, and at the end of intervention (± 3 weeks from Visit 3) as specified in section 7.1 Schedule of Events. Image evidence of cirrhosis and HCC will be recorded and followed. These are standard-of-care tests which are usually performed at 6-month intervals in participants with cirrhosis.

Standard Laboratory Tests
Standard laboratory samples for this study will be assessed using the certified laboratories at the investigators' institutions, or at a clinical laboratory such as Quest or LabCorp. The PI or sub-investigator will review, initial, and date all laboratory results. Any laboratory value outside the reference range that is considered clinically significant by the investigator will be followed as appropriate. Clinically significant laboratory values will be recorded as adverse events if they meet the criteria as specified in Adverse Events (NCI-CTCAE) version 4.0).

8. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

8.1 Primary Endpoint

8.1.1 To establish maximum tolerated dose (MTD) and to collect safety data of Polyphenon E/EGCG treatment in participants with cirrhosis

The safety data of Polyphenon E treatment in participants with liver cirrhosis will be collected at the monthly visit for the first 3 cycles, then at the end of treatment visit for 4th to 6th cycle of treatment). Safety data on hepatotoxicity will be obtained by weekly or bi-weekly (depending on assigned dose, clinical stability, and liver biochemical stability of the participant) liver function tests. Participants who experience hepatotoxicity according to Appendix D will be re-evaluated every 3-4 days until resolution.

Safety and adverse event data will be summarized using descriptive (frequency, %) statistics by dose cohort. The primary endpoint of MTD cannot be established because the study was closed early, and, based on the observed safety profile with a single DTC occurring in dose cohort 4 (1/4 patients), future studies can consider dose level 3 as the starting dose.

8.1.2 To determine the effects of Polyphenon E/EGCG treatment on the suppression of γ-hydroxy-1,N(2)-propanodeoxyguanosine (γ-OHPdG) levels in cirrhotic liver.

Our preliminary data shows that γ-OHPdG predicts HCC development. Among the 3 anti-oxidants—Theaphenon E, lipoic acid, and vitamin E—used in 3 HCC animal models, only Theaphenon E effectively suppressed the accumulation of γ-OHPdG and prevented HCC development. Theaphenon E contains the same active components—epigallocatechin gallate (EGCG), epigallocatechin (EGC), and epicatechin (EC)—as the dietary antioxidant, Polyphenon E. EGCG has been tested in many clinical cancer prevention trials. We propose that the accumulation of the DNA adduct, γ-OHPdG, predicts the development of HCC, and that Polyphenon E suppresses the formation of γ-OHPdG, which may reduce HCC development in participants with cirrhosis. In this pilot study, our primary endpoint is to determine the effect of EGCG treatment on γ-OHPdG levels in cirrhotic liver. During screening, subjects will have a baseline biopsy taken. Only subjects found to have high γ-OHPdG levels (IHC score equal or higher than 3) in their cirrhotic liver will receive EGCG treatment for 24 weeks. An exit liver biopsy will be taken at the end of intervention and will be compared with the baseline biopsy for changes in liver γ-OHPdG levels. All liver tissue slides from the biopsy will be processed and stained with an anti- γ-OHPdG antibody in the lab of Dr. Fung-lung Chung. Two pathologists: Dr Bhaskar Kallakury from GU and Dr. Carmen Gonzalez Keelan from UPR will review the slides and score the level of γ-OHPdG in the participant liver biopsy samples.

8.2 Secondary Endpoints

8.2.1 To collect Polyphenon E/EGCG pharmacokinetic data in participants with cirrhosis.

In previous preclinical studies, the pharmacokinetics of the conjugated metabolites of EGC and EC were not affected by repeat green tea polyphenol daily treatment for 4 weeks. A limited sampling strategy was developed to predict EGCG pharmacokinetics after green tea administration (38). The median sampling times were 0.7, 1.4, and 7.0 hours (fasting conditions) and 1.4, 3.6, and 8.7 hours (fed conditions). The sampling schemes were accurate and precise in predicting EGCG

oral clearance and exposure (area under the curve [AUC]) under both fasting and fed conditions. In this current study, blood and urine samples will be collected in six subjects on day 1 of a 4 week cycle, prior to the first dose of Polyphenon E, and then at 1.5, 3.5, and 8.5 hours (+/- 5 minutes) after the first dose of Polyphenon E, taken while fed. Clearance of Polyphenon E will be compared among cirrhotic liver participants in this study and results from this population will be compared with non-cirrhotic historical control participants (38).

8.2.2 LC-MS assay will be used to determine gamma-OHPdG changes from baseline to post-treatment. This will generate a continuous variable for values of gamma-OHPdG. Descriptive statistics (mean, range) will be used to summarize this continuous outcome by dose level. A nonparametric trend test will be used to examine whether there is a trend for greater reduction in gamma-OHPdG as the dose level increases.

8.2.3 To estimate the fraction of participants with liver cirrhosis that have high levels of γ-OHPdG.

The fraction of participants with liver cirrhosis that have high levels of γ-OHPdG is calculated based on the number of subjects enrolled over the number of subjects screened based on the levels of γ-OHPdG in their first liver biopsy.

8.2.4 Exploratory Endpoint: To assess the effects of Polyphenon E on the grade of cirrhosis as measured by FibroScan and FIB-4 score.

Whether Polyphenon E treatment affects the level of cirrhosis will be evaluated by comparing the reading of FibroScan at baseline and 6 months of EGCG treatment in the subjects participating the intervention study.

8.2.5 Exploratory Endpoint: To develop an LC-MS and/or ELISA-based method for detecting γ-OHPdG.

The research urine samples at Screen 2 and research blood samples collected at Visit 1, Visit 2, and Visit 3 will be used to develop a non-invasive LC-MS and/or ELISA-based method to quantify γ-OHPdG in urine and blood samples of collected from trial participants.

8.2.6 Exploratory Endpoint: To evaluate any HCC development during the treatment

The potential incidence of HCC for participants receiving Polyphenon will be evaluated using ultrasound/CT/MRI performed before and after study intervention.

8.3 Off-Agent Criteria

Participants may stop taking study agent for the following reasons: completed the protocol-prescribed intervention, AE or serious adverse event (SAE) (see section 5.6, Appendix D), inadequate agent supply, noncompliance, concomitant medications, or medical contraindication. Participants will continue to be followed, if possible, for safety reasons and in order to collect endpoint data according to the 7.1 SCHEDULE OF EVENTS.

For any non-liver related toxicities, Polyphenon E will be held for grade 2 adverse events attributed by the treatment until the adverse reaction resolves or improves to baseline, at which time Polyphenon E can be re-initiated at the same dose level. If the adverse reaction re-occurs at grade 2, Polyphenon E should be permanently discontinued.

Hepatotoxicity Assessment Algorithm (**Appendix D**) is being used for hepatotoxicity safety monitoring, in conjunction with NCI CTCAE which is used for grading of adverse events. We will monitor participants' laboratory parameters every 1-2 weeks as recommended, according to protocol section 7. Regarding hepatotoxicity, the procedures for laboratory monitoring, treatment interruption, preinitiation, or permanent discontinuation are described in detail in Appendix D.

If the participant meets at least one criterion of Appendix D, but close monitoring of a participant is not possible, the participant will be taken off agent.

If the participant meets any Discontinue Therapy Criteria, the participant will be taken off agent. DTC is defined as:

a. Grade 3 or higher toxicity based on CTCAE criteria which is possibly, probably, or definitely related to the study agent OR
b. Requires discontinuation of treatment based on the Hepatotoxicity Assessment Algorithm (Appendix D) OR
c. Any hepatic decompensation such as
    1. New onset ascites or worsening of ascites (requiring increase in drug therapy or requiring surgical intervention such as paracentesis).
    2. Variceal bleeding documented by endoscopy
    3. Spontaneous bacterial peritonitis documented by positive culture
    4. Hepatic encephalopathy
    5. Hepatorenal syndrome (Type 1 or 2)
    6. Porto-pulmonary hypertension
    7. Hepato-pulmonary hypertension
    8. Any liver related event that leads to hospitalization or G4 event.

Participants taken off-agent due to adverse event will be followed until resolution of the adverse event or until stabilization of labs and the participant's clinical picture.

8.4 Off-Study Criteria

Participants may go 'off-study' for the following reasons: the protocol intervention and any protocol-required follow-up period is completed, AE/SAE, lost to follow-up, non-compliance, concomitant medication, medical contraindication, withdrawal of consent, death, determination of ineligibility (including screen failure), or pregnancy.

8.5 Study Termination

NCI, DCP as the study sponsor has the right to discontinue the study at any time.

The study will be placed on hold if at any time one of the following occurs:

- One fatality deemed to be possibly, probably, or definitely related to treatment
- ≥ 2 participants meet the criteria for hepatic decompensation, defined as any of the following:
    - New onset ascites or worsening of ascites (requiring increase in drug therapy or requiring surgical intervention such as paracentesis)
    - Variceal bleeding documented by endoscopy
    - Spontaneous bacterial peritonitis documented by positive culture
    - Hepatic encephalopathy
    - Hepatorenal syndrome (Type 1 or 2)
    - Porto-pulmonary hypertension
    - Hepato-pulmonary hypertension
    - Any liver-related event that leads to hospitalization or a grade 4 event
- ≥ 4 participants meet at least one criterion in the algorithm for hepatoxicity (**Appendix D**)

While the study is on hold, the occurrence will be reviewed and investigated by NCI, DCP and Protocol PI and discussed with the FDA to determine whether the study will be terminated. In the dose escalation cohorts, we plan to follow the 3+3 design to avoid unnecessary exposure of participants to EGCG at doses that may cause hepatotoxicity.

9. CORRELATIVE/SPECIAL STUDIES

9.1 Rationale for Methodology Selection

Our proposed study will assess whether our preclinical finding that Polyphenon E decreases the levels of γ-OHPdG holds true in the clinical setting. Our long term goal is to evaluate the role of γ-OHPdG as a predictive biomarker of HCC, and provide a novel prevention strategy for liver cancer, the third leading cause of cancer-related death globally, while expanding knowledge of the antioxidant effects of green tea polyphenols in the prevention of liver cancer.

To evaluate the change of γ-OHPdG in subjects treated with Polyphenon E, two methods will be used to measure the level of γ-OHPdG in liver tissue. First, γ-OHPdG levels in liver specimens will be determined by IHC, as previously described (34). Second, portions of the liver sample will be used for DNA isolation and further assay using LC/tandem MS methodology (for confirmation of γ-OHPdG antibody staining results by IHC) (34). The first method will provide rapid and semi-quantitative measurement of the γ-OHPdG level. The second method will provide quantitative and important confirmatory measurements of γ-OHPdG. IHC analysis is applicable in large-scale analysis of clinical samples, while LC/tandem MS methodology is costly and time consuming, but needed for validation of our IHC assay results. LC/tandem MS is suitable for small-scale sample analysis in a basic research laboratory. In this study, we propose to use LC/tandem MS methodology to confirm the findings from IHC analysis.

Blood and urine will be collected on Visit 1 for measuring bioactive catechin concentrations (EC, EGC, and EGCG) for pharmacokinetics study using a well-developed method (38). In our study, blood and urine samples will be collected in six subjects on day 1 of a 4 week cycle, prior to the first dose, and then at 1.5, 3.5, and 8.5 hours (+/- 5 minutes) after the first dose of Polyphenon E. Clearance of Polyphenon E will be compared among cirrhotic liver of participants in this study

and results from this population will be compared with non-cirrhotic historical control participants (38).

To evaluate the relationships of γ-OHPdG levels in the liver with that found in the urine and blood samples of these participants, urine samples will be collected at Screen 2 from participants who are subjected to a liver biopsy. Blood samples will be collected for all study participants at Visit 1, Visit 2, and Visit 3. As a non-invasive method is a prerequisite for the clinic application of γ-OHPdG as a prognostic biomarker, we will make an effort to develop a non-invasive method for measuring γ-OHPdG in urine and blood. The hypothesis is that the urinary γ-OHPdG reflects its hepatic levels. An LC-MS/MS and/or ELISA based method will be developed and the correlation coefficient between urinary, blood, and liver γ-OHPdG levels will be established.

Compliance will be verified by using the urine samples collected at Visits 2 and 3. These samples will be analyzed for green tea catechins, specifically EGCG, EGC and EC by the HPLC-EC method described in literature which was further developed in Dr. Chung's lab.

Studies have shown that γ-OHPdG adduct formation causes GC to TA and GC to AT mutations (12,13). In our preclinical HCC animal model, from whole exome next-generation sequencing, we found GC to TA mutation as the dominant alteration, accounting for 92% and 86% mutations, respectively. The high GC to TA mutation frequency suggests that γ-OHPdG plays a role in the mutagenesis that leads to HCC development. DNA sequencing of human liver biopsy samples will allow us to assess changes in the mutation spectrum in liver biopsies before and after Polyphenon E treatment. This information is critical to our ability to assess the role of γ-OHPdG DNA adduct formation in the development of liver cancer in our currently proposed biomarker driven study. β-Catenin and p53 are the two most frequently mutated genes in HCC. If we are able to shown Polyphenon E treatment decreases the accumulation of γ-OHPdG, and also decreases the mutations frequency in p53, and β-catenin (CTNNB1) gene, the data will support the mechanistic role of γ-OHPdG in hepatocarcinogenesis, and Polyphenon E as an intervention for HCC prevention. The data from this pilot study will support a larger study evaluating Polyphenon E as a preventive treatment for HCC.

9.2 Comparable Methods

In this study, we use IHC, a semi-quantitative method to measure the levels of γ-OHPdG in body tissues, and LC/tandem MS methodology, a quantitative method, to confirm these of γ-OHPdG levels. There are no other methods used to measure γ-OHPdG levels. Only one such comparison was reported (34) and we will compare the results from this study to those previously reported. We found that approximately 30% of cirrhotic participants expressed high levels of γ-OHPdG in their liver biopsies. We will be able to compare this previous observation with the data obtained in this current study.

The methods used for pharmacokinetic study and gene target sequencing are well-established procedures. No better methods exist for obtaining the data needed for this study.

The novelty of the study lies in our evaluation of the change of γ-OHPdG levels in participants treated with Polyphenon E using standard established methods.

β-Catenin and p53 are the two most frequently mutated genes in HCC. Obtaining the mutation spectrum of β-Catenin and p53 is sufficient to evaluate the change of GC to TA and GC to AT mutations prior to and after Polyphenon E treatment. Measurement of the mutation spectrum of specific genes is less costly compared to whole genome sequencing.

10. SPECIMEN MANAGEMENT

10.1 Laboratories

EGCG, EC, and EGC concentrations in plasma and urine samples are determined within one month of collection. This polyphenol analysis will take place in the laboratory of Dr. Fung-Lung Chung. Dr. Chung's lab is located at Georgetown University Medical Center, New Research Building E215, Washington, DC 20007-2126, Tel: (202)-687-3068.

Pharmacokinetic analysis of EGCG level in blood and urine samples collected from participants receiving Polyphenon E treatment will be carried out at North Carolina Agricultural and Technical State University. The lab is located at North Carolina A&T State University Research Campus Suite 4345, UNC Nutrition Research building, 500 Laureate Way, Kannapolis, NC 28081.

Measurement of γ-OHPdG levels in liver specimens by IHC will be carried out by Dr. Brent Harris at Histopathology and Tissue Shared Resource of Lombardi Comprehensive Cancer Center (LCCC) located at LR-10 Pre-Clinical Sciences Building at LCCC. The antibody used for the IHC assay will be provided by Dr. Fung-Lung Chung. The IHC slides will be reviewed at the departments of pathology at GUH and UPR. After the slides have been reviewed by Dr. Bhaskar Kallakury at GUH, they will be shipped to Dr. Carmen Gonzalez Keelan at the department of pathology at UPR for review.

Quantitative measurement of γ-OHPdG in liver by DNA isolation, and further assay using LC/tandem MS methodology will be carried out at the University of Minnesota. The lab is located at Masonic Cancer Center University of Minnesota, Cancer and Cardiovascular Research Building, 2231 6th Street SE, Minneapolis, MN 55455.

10.2 Collection and Handling Procedures

Study Time Points				
Procedure	**Screen 2 (0-60 days prior to registration)**	**Visit 1: Week1 Day 1 (0-14 days after registration)**	**Visit 2: Week 13 +/- 3 days)**	**Visit 3: End of Treatment (week 25 +/- 3 days)**
Biopsy: Formalin Fixed Flash Frozen (DMSO Solution)	x*			x
Research Blood – γ-OHPdG Buffy coat --IHC Plasma --Affinity Column Based LC-MS/MS or ELISA		x	x	x
Research Blood – γ-OHPdG in DNA for LC-MS/MS and ELISA		x	x	x

Research Urine – γ-OHPdG	x			
Research Urine - Compliance			x	x
Research Blood PKs** collected on Visit 1 (pretreatment, 1.5, 3.5, and 8.5 hours (+/- 5 minutes) post treatment)		x		
Research Urine PKs** (self-collected at pretreatment, 1.5, 3.5, and 8.5 hours (+/- 15 minutes) post treatment)		x		
* Liver biopsy is required for participants who have no archival liver biopsy tissue adequate for γ-OHPdG testing collected and stored within 12 months prior to consent. Participants with archival liver biopsy tissue adequate for γ-OHPdG testing, and collected and stored within 12 months prior to consent, may optionally have the liver biopsy at Screen 2. If the participant has archival liver biopsy tissue collected within the last 12 months prior to consent, the archival tissue can be tested (or sent for testing) for γ-OHPdG at either Screen 1 or Screen 2. Archival tissue will be sent to Dr. Chung's laboratory as a formalin-fixed, paraffin-embedded block. ** Research Urine and Research Blood PKs are performed on the first six participants at Georgetown University who agree to provide extra blood and urine.				

Liver Biopsies: Screen 2 and Visit 3

Liver biopsy is required at Screen 2 for participants who have no archival liver biopsy tissue adequate for γ-OHPdG testing collected and stored within 12 months prior to consent. Participants with archival liver biopsy tissue adequate for γ-OHPdG testing, and collected and stored within 12 months prior to consent, may optionally have the liver biopsy at Screen 2. All participants will have a liver biopsy at Visit 3, regardless of archival tissue availability.

Biopsies will be performed as per standard of care at the sites to generate one to two cores (diameter about 2 mm, with a throw length of approximately 2 cm), which will provide adequate tissue for study. Each set of core biopsies will be divided and processed as below for later analysis:

1) Formalin-fixed, paraffin-embedded (FFPR) block
    a. Place the 1/4th core in the middle of the bottom Telfa paper, put the top piece of Telfa paper back to cover the biopsy specimen.
    b. Close the cassette and put it into 10% neutral buffered formalin and fix for 12 hours.
    c. Store the specimen in 70% ethanol and make paraffin block.
    d. Each mold will be labeled with the specimen ID number.
    e. The formalin fixed core will be shipped at 4°C (25% of the specimen, 1/4 core biopsy).
2) Flash-frozen
    a. Transfer the remaining ¾ core into a separate 4mL cryovial or 100mL biopsy cup with DSMO-salt solution.
    b. Screw the cap of specimen, place study label to the tubes, and then place each sample in liquid nitrogen.
    c. Once cryogenic molds are frozen, transfer them to dry ice and store samples at -80°C.

Archival Liver Biopsy Tissue: Screen 1 or Screen 2

If the participant has archival liver biopsy tissue collected within the last 12 months prior to consent, the archival tissue can be tested (or sent for testing) for γ-OHPdG at either Screen 1 or Screen 2. Archival tissue will be sent to Dr. Chung's laboratory as a formalin-fixed, paraffin-embedded block. If archival tissue is used in lieu of a Screen 2 liver biopsy (fresh tissue), then the pre-study p53 and β-catenin analyses will be performed on the formalin-fixed, paraffin-

embedded archival tissue block (instead of using frozen fresh tissue).

Research Urine: Screen 2, Visit 2, and Visit 3
At Screen 2, Research Urine for measuring γ-OHPdG should be collected prior to the Screen 2 Liver Biopsy for participants. At Visit 2 and Visit 3, compliance to treatment will be assessed using urine samples.

For Screen 2 urine collection (γ-OHPdG):

1) Instruct the participant to:
    a. Wash hands with soap and warm water.
    b. Hold the 100 mL Urine Specimen Cup labeled "Urine" as the specimen type, a few from the urethra and urinate, collection the first-void urine until the cup is half full.
    c. Void the rest of the urine into the toilet.
    d. Tightly screw the cap onto the cup and place in the biohazard transport bag
    e. Clean-up any spills on the cup using the provided towelette.
2) Place the pre-generated label noting the participants study ID to the urine cup.
3) For MedStar Georgetown: transfer urine specimen to the Dr. Chung's laboratory for processing along with the processing labels and collection form.
4) For non-MedStar Georgetown University sites: freeze and store samples immediately at -80°C or colder.

For Visit 2, and Visit 3 urine collection (compliance):

1) Two urine collection cups will be provided in the kit, only the 100ml urine cup labeled "Parent Tube" will be given to the participant. Place the pre-generated label noting the participants study ID to the urine cup.
2) Instruct the participant to:
    a. Wash hands with soap and warm water.
    b. Hold the 100 mL Urine Specimen Cup labeled "Urine" as the specimen type, a few from the urethra and urinate, collection the first-void urine until the cup is half full.
    c. Void the rest of the urine into the toilet.
    d. Tightly screw the cap onto the cup and place in the biohazard transport bag
    e. Clean-up any spills on the cup using the provided towelette.
3) Transfer 30ml of urine sample to 30ml vial labeled "daughter tube" and mix with powder within 20 minutes of sample collection or as soon as possible. Place the pre-generated label noting the participants study ID to the urine cup.
4) For MedStar Georgetown: transfer urine specimen to the Dr. Chung's laboratory for processing. Along with the processing labels and collection form.
5) For non-MedStar Georgetown University sites: freeze and store samples immediately at -80°C or colder.

Research Blood: Visit 1, Visit 2, and Visit 3
Research Blood is taken at Visit 1, Visit 2, and Visit 3. The Research Blood on Visit 1 will be taken prior to the first dose of study agent.

2 x 6mL NaHep (Green Top) Tubes - γ-OHPdG research blood

1) Collect blood until vacuum is exhausted.
2) After blood collection, gently invert tubes 8-10 times until blood is completely mixed

3) Place pre-generated label containing participants ID to each tube
4) Place tubes in an upright position immediately on wet ice to then transfer over to lab for processing
5) Within 2 hours of collection, centrifuge tubes at 2200-2500 RPM for 15 minutes at 4°C
6) Remove plasma (supernatant) carefully and do not disturb the thin layer of white cells between the plasma and the red blood cells. Save tube for buffy coat processing.
7) Distribute the plasma into 2ml screw topped cryogenic vials. Place 1mL (minimal 0.5mL, maximal 1.5mL) of plasma in each aliquot. 6 aliquots will be collected (3 cryovials of plasma from each parent tube).
8) Buffy coat will be isolated and transferred into 2mL cryogenic vial, then flash frozen and stored at -80°C.
9) Place pre-generated label containing participants ID to each cryovial, ensuring that daughter specimen labels match the parent specimen labels used on the tubes.
10) Plasma aliquots need to be frozen in -80°C immediately

<u>2 x 6mL EDTA (Purple Top) Tubes - γ-OHPdG Whole Blood for LC-MS/MS and ELISA</u>

1) Collect blood until vacuum is exhausted.
2) After blood collection, gently invert tubes 8-10 times until blood is completely mixed.
3) Place pre-generated label containing participants ID to each tube.
4) Store at ambient temperature until shipping.

Research Blood PKs: Visit 1 (week 1 day 1) only

Six participants at Georgetown University will participate in a PK evaluation. The day before the pharmacokinetic study (Visit 1), Georgetown University study participants are instructed to fast after midnight except for drinking water. On the pharmacokinetic study day, Georgetown University study subjects skip breakfast and take no over the counter medications, vitamins, or health food products. Georgetown University study participants come to the clinic in the early morning (6–8 a.m.) and are provided with food for breakfast. Immediately after or during breakfast, study subjects swallow their Polyphenon E capsules with a glass of water. Study subjects are allowed unlimited water intake throughout the study day. Other drinks are not allowed. They will fast until they take their first dose of Polyphenon E. Blood will be collected:

- Prior to the first dose
- 1.5 hours (+/- 5 minutes) after first dose
- 3.5 hours (+/- 5 minutes) after first dose. Food for lunch is provided to the study subjects after this blood draw.
- 8.5 hours (+/- 5 minutes) after first dose

For each blood draw:

1) Draw 4 mL of whole blood in each of two green-top tubes containing sodium heparin (for a total of 8 mL of whole blood).
2) Collect blood until vacuum is exhausted for each tube.
3) After blood collection, gently invert tubes 8-10 times until blood is completely mixed.
4) Record the time of the dose administration and the time of the blood draw.
5) Place pre-generated label containing participants ID to each tube
6) After collection, place tube in an upright position immediately on wet ice.
7) Within 2 hours of collection, centrifuge tubes at 2000-2500 RPM for 15 minutes at 4°C.

8) Distribute the plasma into 2mL screw topped cryogenic vials. Place 1ml (minimal 0.5ml, maximal 1.5ml) of plasma in each aliquot. 4 aliquots will be collected (2 cyrovials of plasma from each parent tube). Note: Do not transfer red cells to the vial.
9) Add 20 ul of ascorbic-EDTA solution [0.4 m NaH2PO4 buffer containing 20% ascorbic acid and 0.1% EDTA (pH 3.6)], screw the top and gently mix.
10) Place pre-generated label containing participants ID to each cryovial, ensuring that daughter specimen labels match the parent specimen labels used on the tubes.
11) Freeze and store samples at −80°C until analysis or shipment.

Research Urine PKs: Visit 1 (week 1 day 1) only
Six participants at Georgetown University will participate in a PK evaluation. They will fast until they take their first dose of Polyphenon E. They will take their first dose of Polyphenon E with food, and urine (30 ml) will be collected:

- Prior to the first dose
- 1.5 hours (+/- 15 minutes) after first dose
- 3.5 hours (+/- 15 minutes) after first dose
- 8.5 hours (+/- 15 minutes) after first dose

For urine collection (PK):

1) Two urine collection cups will be provided in the kit, only the 100ml urine cup labeled "Parent Tube" will be given to the participant. Place the pre-generated label noting the participants study ID to the urine cup.
2) Instruct the participant to:
    a. Wash hands with soap and warm water.
    b. Hold the 100 mL Urine Specimen Cup labeled "Urine" as the specimen type, a few from the urethra and urinate, collection the first-void urine until the cup is half full.
    c. Void the rest of the urine into the toilet.
    d. Tightly screw the cap onto the cup and place in the biohazard transport bag
    e. Clean-up any spills on the cup using the provided towelette.
3) Transfer 30ml of urine sample to 30ml vial labeled "daughter tube" and mix with powder within 20 minutes of sample collection or as soon as possible. Place the pre-generated label noting the participants study ID to the urine cup.
4) For MedStar Georgetown: transfer urine specimen to the Dr. Chung's laboratory for processing. Along with the processing labels and collection form.
5) For non-MedStar Georgetown University sites: freeze and store samples immediately at -80°C or colder.

10.3 Shipping Instructions

All samples will be shipped in compliance with the International Air Transport Association Dangerous Goods Regulations. All shipments should be batch shipped once a week, Monday through Friday, after communication with the receiving site.

Whole blood and FFPE biopsy specimens (including archival biopsy samples) will be shipped at refrigerated temperature (4°C). All other specimens (the remaining two biopsy samples, Research

Plasma, Research Blood PKs, and Research Urine PKs) will be packed in sufficient dry ice to last during transport for 3 days, and shipped or transported from the study site to:

Dr. Fung-Lung Chung
3970 Reservoir Road, NW
Georgetown University Medical Center
New Research Building E215
Washington, DC 20057
Tel: 202-687-3021
Fax: 202-687-1068
Email: flc6@georgetown.edu

10.4 Tissue Banking

The biopsy samples will be labeled appropriately with the study protocol number, subject number, the date of collection, study time point, and location of collection (GUH or UPR) and stored at Dr. Chung's laboratory in a -80^{o} Freezer. FFPE biopsy specimens will be stored at room temperature.

Biological specimens collected during the conduct of each clinical trial that are not used during the course of the study will be considered deliverables under the contract and thus the property of the NCI. At study completion, NCI reserves the option to either retain or relinquish ownership of the unused biologic specimens. If NCI retains ownership of specimens, the Contractor shall collect, verify and transfer the requested biologic specimens from the site to a NCI-specified repository or laboratory at NCI's expense.

11. REPORTING ADVERSE EVENTS

DEFINITION: AE means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can therefore be any unfavorable and unintended sign), 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.

Please note that all abnormal clinical laboratory values that are determined to be of clinical significance based on a physician's assessment are to be reported as AEs. Those labs determined to be of no clinical significance or of unknown clinical significance (per the physician's assessment) should not be reported as AEs. Any lab value of unknown clinical significance should continue to be investigated/followed-up further for a final determination, if possible.

A list of AEs that have occurred or might occur can be found in §6.2 Reported Adverse Events and Potential Risks, as well as the Investigator Brochure or package insert.

11.1 Adverse Events

11.1.1 Reportable AEs

All AEs that occur after the informed consent is signed and baseline assessments are completed must be recorded on the AE CRF (paper and/or electronic) whether or not related to study agent.

11.1.2 AE Data Elements:

The following data elements are required for AE reporting.

- AE verbatim term
- NCI Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0) AE term (MedDRA lowest level term)
- CTCAE (MedDRA) System Organ Class (SOC)
- Event onset date and event ended date
- Treatment assignment code (TAC) at time of AE onset
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as a SAE
- Whether or not the subject dropped due to the event
- Outcome of the event

11.1.3 Severity of AEs

11.1.3.1 Identify the AE using the CTCAE version 4.0. The CTCAE provides descriptive terminology (MedDRA lowest level term) and a grading scale for each AE listed. A copy of the CTCAE can be found at
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

AEs will be assessed according to the grade associated with the CTCAE term. AEs that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4.0 as stated below.

CTCAE v4.0 general severity guidelines:

Grade	Severity	Description
1	Mild	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
4	Life-threatening	Life-threatening consequences; urgent intervention indicated.
5	Fatal	Death related to AE.

ADL

*Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, *etc*.

**Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

11.1.4 Assessment of relationship of AE to treatment

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

11.1.5 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.

11.2 Serious Adverse Events

11.2.1 DEFINITION: Regulations at 21 CFR §312.32 (revised April 1, 2014) defines an SAE as any untoward medical occurrence that at any dose has one or more of the following outcomes:

- Death
- A life-threatening AE
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to perform normal life functions
- A congenital anomaly or birth defect
- Important medical events that may not be immediately life-threatening or result in death or hospitalization should also be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient and may require intervention to prevent one of the other outcomes.

11.2.2 Reporting SAEs to DCP

11.2.2.1 The Lead Organization and all Participating Organizations will report SAEs on the DCP SAE Report Form found at http://prevention.cancer.gov/clinical-trials/clinical-trials-management/protocol-information-office/pio-instructions-and-tools/2012-consortia.

11.2.2.2 Contact the DCP Medical Monitor by phone within 24 hours of knowledge of the event.

DCP Medical/Task Order Monitor
Luz Maria Rodriguez, MD, FACS
NCI/Division of Cancer Prevention
9609 Medical Center Dr.

Rm 5E-228
Bethesda, MD 20892
Telephone (240) 276-7039
Fax (240) 276-7848
Email: rodrigul@mail.nih.gov

Include the following information when calling the Medical Monitor:

- 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
- DCP protocol number
- Title of protocol
- Description of the SAE, including attribution to drug

11.2.2.3 The Lead Organization and all Participating Organizations will email written SAE reports to the following within 48 hours of learning of the event, using the fillable PDF SAE Report Form.

- DCP's Regulatory Contractor CCS Associates, Inc. (CCSA; phone: 650-691-4400) at safety@ccsainc.com
- DCP Medical Monitor, Dr. Luz Rodriguez (rodrigul@mail.nih.gov)
- Northwestern Cancer Prevention Consortium (ncpc@northwestern.edu)
- The Protocol PI, Dr. Ruth He (arh29@georgetown.edu)

11.2.2.4 The DCP Medical Monitor and CCSA regulatory and safety staff will determine which SAEs require FDA submission as IND safety reports.

11.2.2.5 The Lead Organization and all Participating Organizations will comply with applicable regulatory requirements related to reporting SAEs to the IRB/IEC.

11.2.3 Follow-up of SAE

Site staff should send follow-up reports as requested when additional information is available. Additional information should be entered on the DCP SAE Report Form in the appropriate format. Follow-up information should be sent to DCP as soon as available. The length of time for follow-up of an SAE is 6 months.

12. STUDY MONITORING

12.1 Data Management

Data will be managed by the study statistician, Dr. Kocherginsky, according to standard operating procedures, which meet the guidelines of DCP Requirements for Data Management and which follow the Data Management Plan that Northwestern University has on file with the Division of Cancer Prevention, NCI. The Consortia 2012 Data Management Plan, submitted as part of a

contract agreement with the NCI (HHSN261201200035I), was approved.

12.2 Case Report Forms

Participant data will be collected using protocol-specific case report forms (CRFs) developed from the standard set of DCP Chemoprevention CRF Templates and utilizing NCI-approved Common Data Elements (CDEs). The approved CRFs will be used by Northwestern University to create the electronic CRF (e-CRF) screens in the Robert H. Lurie Comprehensive Cancer Center of Northwestern University (Lurie Cancer Center) Clinical Trials Management System (CTMS). Accrual-site staff will enter data into the e-CRFs for transmission to DCP according to DCP standards and procedures.

12.3 Source Documents

All source documents will be collected and stored in the Clinical Research Office of the site where the participant was accrued. Any data recorded directly on CRFs that constitute no prior written or electronic record of data, will be specifically identified as source data.

12.4 Data and Safety Monitoring Plan

A comprehensive Data Safety and Monitoring Plan has been submitted by Northwestern University, approved by the DCP, and is on file there. This study will be subject to Northwestern Data Monitoring Committee review according to the Master DSMP. Any future changes will be forwarded for review.

As part of our Master DSMP, this study will be reviewed semi-annually by a Data Monitoring Committee. This semi-annual report includes a list of all reported adverse events, serious adverse events, dose limiting toxicities, and protocol deviations.

Adverse events are reviewed monthly by the NCI DCP medical monitor through the submission of the Minimum Data Set.

12.5 Sponsor or FDA Monitoring

The NCI, DCP (or their designee), pharmaceutical collaborator (or their designee), or FDA may monitor/audit various aspects of the study. These monitors will be given access to facilities, databases, supplies and records to review and verify data pertinent to the study.

12.6 Record Retention

Clinical records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, *etc*.), as well as IRB records and other regulatory documentation will be retained by the Investigator in a secure storage facility in compliance with Health Insurance Portability and Accountability Act (HIPAA), Office of Human Research Protections (OHRP), Food and Drug Administration (FDA) regulations and guidances, and NCI/DCP requirements, unless the standard

at the site is more stringent. The records for all studies performed under an IND will be maintained, at a minimum, for two years after the approval of a New Drug Application (NDA). For NCI/DCP, records will be retained for at least three years after the completion of the research. NCI will be notified prior to the planned destruction of any materials. The records should be accessible for inspection and copying by authorized persons of the Food and Drug Administration. If the study is done outside of the United States, applicable regulatory requirements for the specific country participating in the study also apply.

12.7 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA)

N/A

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Description

We propose a single-arm, multicenter MTD seeking, escalation study. To meet our primary goal, 2-48 participants with cirrhotic livers expressing high levels (score 3 and above) of γ-OHPdG (γ-OHPdG-high) in their baseline biopsy are required for the Polyphenon E intervention portion of the study.

In our unpublished data, 68 participants with cirrhosis, 38% (90% confidence interval, range 0.28, 0.49) of cirrhotic liver biopsies demonstrated high γ-OHPdG levels (IHC score ≥ 3). Therefore, a minimum of 4 and maximum of 171 participants with liver cirrhosis will be screened in our current study. A minimum of 2 and a maximum of 48 participants whose cirrhotic liver biopsies show a γ-OHPdG score ≥ 3 will be registered for treatment. If a maximum tolerated dose is established, a total of 24 participants will receive the maximum tolerated dose. The maximum tolerated dose is defined as the highest dose where no more than 1 of 6 participants experience an event meeting the Discontinue Therapy Criteria (DTC) during the first four weeks of treatment and the next higher dose is such that 2 or more participants had experienced an event meeting the DTC during the first four weeks of treatment.

Trial enrollment was stopped early, with 13 patients enrolled in Cohorts 1- 4 during dose escalation, of whom 10 participants had both pre- and post-treatment biopsies for biomarker assessment (Table 6). No participants were enrolled in the dose expansion cohort.

Table 6: Trial Enrollment

Cohort	Started Trt	Pre & Post Bx	DTC
Cohort 1 (400mg QD)	3	3	0
Cohort 2 (400 mg BID)	3	3	0
Cohort 3 (600 mg BID)	3	2	0
Cohort 4 (800 mg BID)	4	2	1

13.2 Randomization/Stratification

There is no randomization in this study.

13.3 Accrual and Feasibility

The following strategies will be applied to ensure complete enrollment within the first twelve months of study initiation:

MedStar Georgetown Transplant Institute is a network of multidisciplinary teams that provides medical care to participants with chronic liver disease. This network includes GUH, Washington Hospital Center (WHC), Franklin Square Medical Center, and MedStar Georgetown Transplant Institutes in Fairfax and Frederick. The network serves about 2,200 participants with chronic liver disease each year. Drs. Smith, Dr. Rangnekar, and Dr. Thomas have weekly clinics in MedStar GUH, WHC, and Georgetown Transplant Institute in Fairfax. When they identify potential candidates at any of the sites (pre-screen), these participants will be screened for the study at MedStar GUH (screen 1 and 2) and registered if indeed eligible. The distance between MedStar Georgetown Transplant Institute in Fairfax and MedStar GUH is 17 miles (a 25 minute drive by car), and the distance between MedStar WHC and GUH is 5 miles (a 15 minute drive by car); these distances allow participants relatively easy travel from MedStar Georgetown Transplant Institute in Fairfax or WHC to GUH for medical care.

Potential candidates from Franklin Square Medical Center and transplant clinic at Frederick will also be referred to GUH for this study. Among the 2,200 hepatology participants seen under the MedStar Network within one year, approximately 20% have compensated cirrhosis without liver cancer. Therefore we have a pool of 440 participants that meet the eligibility criteria for this study. Based on our small 50-patient survey, 90% of participants should be interested in participating in the study, despite having to undergo liver biopsy. Thus we have a pool of 400 participants per year from which to draw potential study candidates.

At UPR, over 1000 participants are seen annually over the hepatology clinical enterprise. It is estimated that approximately 50% of these participants have underlying cirrhosis at different stages, including pre-transplant participants. Dr. Cruz estimates that 10% of cirrhotic individuals may be eligible for the study translating to approximately 50-100 participants providing adequate numbers for screening into the study.

One study coordinator will be shared by Drs. Coleman Smith and the investigator's at Georgetown for participant enrollment and follow-up in Washington DC. One or two specific days of the week can be assigned to each physician for seeing participants enrolled onto this study, so the study coordinator will be able to follow all participants enrolled in Washington DC. Monthly teleconferences will be arranged for Drs. Coleman Smith, Ruth He, and the study coordinators to discuss enrollment and study related issues.

With these prescreening and rolling enrollment strategies, we believe that we will be able to screen 171 participants with liver cirrhosis in order to be able to find 48 participants whose cirrhotic liver biopsies show a γ-OHPdG score ≥ 3 by IHC (γ-OHPdG-high) to be enrolled into the pilot study within the first year.

Our strategies are to prescreen most participants by medical record review after the protocol is approved, obtain consent, and, if needed, obtain a baseline biopsy from most participants within the first few months of study initiation so that candidates for intervention enrollment for are identified quickly. From the pool of study candidates, we plan to enroll 6 participants per month if. With this plan, even if there are delays for any reason, we will be able to complete the study within two years.

We plan to allow up to three weeks from the day of biopsy to obtain the liver γ-OHPdG score. The liver samples will be batched and tested twice per month. If the rate of cirrhotic liver biopsies with a γ-OHPdG score ≥ 3 is lower than expected, we will increase the number of subjects that are pre-screened per month.

13.4 Primary Objective, Endpoint(s), Analysis Plan

The primary objectives are to establish MTD, collect safety data of Polyphenon E/EGCG at MTD treatment in participants with cirrhosis and to determine the suppressing effects of /Polyphenon E/EGCG treatment on γ-hydroxy-1,N(2)-propanodeoxyguanosine (γ-OHPdG)levels in cirrhotic liver.

To assess the safety of Polyphenon E treatment in participants with cirrhosis, we will list all the adverse events and provide summary statistics.

To explore possible effects of Polyphenon E treatment on γ-OHPdG levels in cirrhotic liver samples of participants known to have high levels in liver biopsy samples.

The primary study objective is to assess the effect of Polyphenon E treatment on γ-OHPdG expression. Based on our preliminary data, we expect a 30-40% decrease in γ-OHPdG expression in cirrhotic liver after six months of Polyphenon E treatment. Twenty-four subjects with high γ-OHPdG levels in their liver biopsies will receive Polyphenon E. This provides 80% or greater statistical power to confirm a 30% or bigger decrease in γ-OHPdG levels at a 0.05 alpha level with a two sided test, and coefficient of variation = 50%-100%. We will use a nonparametric Wilcoxon test to compare the post-pre differences to zero. We anticipate that high pre-therapy IHC scores = 3, 4 and 5 vs. post-therapy scores mean = 2, —using a pre-therapy average of 4 to be conservative. Thus we will have two scores per person. The difference between Before and After = Before - After = B-A should be on average = 0 if treatment has no effect and on average about 1.33 and 1.60 after treatment = reduction of 33% and 40% respectively, down from 4.

Under usual assumptions of corr (B,A) = 0.5 and SD(B) = SD(A) we get mathematically SD(B-A) = SD(B) = SD(A) = 2. SD=2 was selected so as to give CV=100% when mean = 2 and CV=50% when mean = 4.

Numeric Results for Wilcoxon Test (Normal Distribution)

Null Hypothesis: Mean0=Mean1 Alternative Hypothesis: Mean0≠Mean1

Unknown standard deviation.

Power	N	Alpha	Beta	Mean0	Mean1	SD	Effect Size
0.84466	24	0.05	0.15534	0	1.33	2	0.665
0.94694	24	0.05	0.05306	0	1.6	2	0.8

Safety and adverse event data will be summarized using descriptive (frequency, %) statistics by dose cohort. The primary endpoint of MTD cannot be established because the study was closed early, and, based on the observed safety profile with a single DTC occurring in dose cohort 4 (1/4 patients), future studies can consider dose level 3 as the starting dose.

13.5 Secondary Objectives, Endpoints, Analysis Plans

Secondary endpoints:

a) To collect Polyphenon E/EGCG pharmacokinetic data in participants with cirrhosis.

b) To determine the effects of Polyphenon E/EGCG treatment on the suppression of γ-hydroxy-1,N(2)-propanodeoxyguanosine (γ-OHPdG) levels in cirrhotic liver by LC-MS assay from baseline to post-treatment.

c) To estimate the fraction of participants with liver cirrhosis that have high levels of γ-OHPdG.

We will test the γ-OHPdG levels at baseline and at the end of study in all subjects. Based on our preclinical models, we believe six months of intervention will decrease the γ-OHPdG levels. We will use two methods to measure the level of γ-OHPdG including IHC stain and Mass Spectrum analysis. The pathology slides prepared from the liver biopsy will be stained for antibody targeting γ-OHPdG. The level of γ-OHPdG will be scored from 0 to 6. IHC score of 0 to 2 is defined as γ-OHPdG low, and IHC score of 3 to 6 is defined as defined as γ-OHPdG high. When γ-OHPdG is measured by mass spectrum, the absolute amount of γ-OHPdG will be measured. In this study, we hope to evaluate whether decrease in γ-OHPdG level will result in decrease in mutation frequency only in five subjects due to budget restriction. The data will help to test our hypothesis and support future studies. If there is no restriction in budget, we would like to carry out the study in all subjects.

For secondary outcome (b) LC-MS assay will be used to determine gamma-OHPdG changes from baseline to post-treatment. This will generate a continuous variable for values of gamma-OHPdG. Descriptive statistics (mean, range) will be used to summarize this continuous outcome by dose level. A nonparametric trend test will be used to examine whether there is a trend for greater reduction in gamma-OHPdG as the dose level increases.

For secondary outcome (c) we will use descriptive statistics to estimate the fraction of participants with liver cirrhosis that have high levels of γ-OHPdG. Participants will be screened for high levels of γ-OHPdG. Only participants with high levels of γ-OHPdG will be enrolled for intervention. The

fraction of participants with liver cirrhosis that have high levels of γ-OHPdG is calculated based on the number of subjects enrolled over the number of subjects screened based on the levels of γ-OHPdG in their first liver biopsy. This important information may help to estimate the portion of cirrhotic participants with high risk for HCC development, and can be used to design a future large chemoprevention study using Polyphenon E or other green tea extract in HCC prevention.

Due to the early stopping, secondary and exploratory endpoints will be summarized using descriptive statistics across dose cohorts. Changes in biomarkers across dose levels will be analyzed using a nonparametric trend test.

Exploratory endpoint:
For exploratory objective, we will assess the effects of Polyphenon E on the grade of cirrhosis, as measured by FibroScan. We will also develop an LC-MS and/or ELISA-based method for detecting urinary and blood γ-OHPdG. Additionally, we will measure the incidence of HCC across all dosage levels.

Descriptive statistics (n; min; max; mean; median; SD for continuous variables; and n, frequency for categorical variables) will be used to summarize participant demographics. Participant safety data will be tabulated according to the symptom and grade. Estimates will be presented with their 95% confidence intervals.

Proposed Sample Size: Final number of n=2 to 48 subjects.
Proposed Sample Size: A minimum of 4 and maximum of 171 eligible cirrhotic participants will be screened. Enrollment of subjects will stop according to the dose escalation 3 + 3 design, plus an expansion cohort of up to 24 subjects at the maximum tolerated dose if one is established, for a total of n = 2 to 48 subjects.

13.6 Reporting and Exclusions

We expect that we will identify an MTD from the 3+3 dose escalation cohort, then move to dose expansion cohort.

We expect that 2 to 48 subjects will have complete data on the primary endpoint. Main analysis will be based on the complete data. Additional descriptive analysis may be performed to see if dropouts show a systematic property. Thus, we expect the 15% of data missing values will be studied to determine possible patterns and dependence with other relevant variables.

We will report the reasons for any missing outcomes to determine if they may be related to the unobserved outcome. We will write a cautionary note that results could be biased if there is a background change in γ-OHPdG over time that is unrelated to Polyphenon E treatment.

13.7 Evaluation of Toxicity

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

13.8 Evaluation of Response

Intent to treat analysis: All samples from all subjects who receive drug will be evaluated and included in the primary analysis, which will be based on intent to treat principle.

13.9 Interim Analysis

In dose escalation cohort, subjects enrolled in each dose level will be monitored for liver toxicities for four weeks prior to enrolling additional participants in the next dose level.

Since all study subjects are planned to be enrolled within 12 months, interim analysis for efficacy will not be performed.

13.10 Ancillary Studies

The distribution of γ-OHPdG in participants with cirrhosis and stability of γ-OHPdG in cirrhotic participants have been studied, and data is provided in section 2.3.4 and 2.3.5 on page 13.

14. ETHICAL AND REGULATORY CONSIDERATIONS

14.1 Form FDA 1572

Prior to initiating this study, the Protocol Lead Investigator at the Lead or Participating Organization(s) 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. All personnel directly involved in the performance of procedures required by the protocol and the collection of data should be listed on Form FDA 1572.

14.2 Other Required Documents

14.2.1 Current (within two years) CV or biosketch for all study personnel listed on the Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations. CVs or biosketches do not need to be updated for participating study staff after drug shipment authorization (DSA).

14.2.2 Current medical licenses (where applicable) for all study personnel listed on Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.3 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.

14.2.4 Documentation of Good Clinical Practice training for all study personnel listed on the FDA Form 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.5 Documentation of Federalwide Assurance (FWA) number for the Lead Organization and all Participating Organizations.

14.2.6 Signed Investigator's Brochure/Package Insert acknowledgement form

14.2.7 Delegation of Tasks form for the Lead Organization and all Participating Organizations signed by the Principal Investigator for each site and initialed by all study personnel listed on the form

14.2.8 Signed and dated NCI, DCP Financial Disclosure Form for all study personnel listed on Form FDA 1572 for the Lead Organization and all Participating Organizations

14.3 Institutional Review Board Approval

Prior to initiating the study and receiving agent, the Investigators at the Lead Organization and the Participating Organization(s) must obtain written approval to conduct the study from the appropriate IRB. Should changes to the study become necessary, protocol amendments will be submitted to the DCP PIO according to DCP Amendment Guidelines. The DCP-approved amended protocol must be approved by the IRB prior to implementation

14.4 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 and date 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.

Participants must be provided the option to allow the use of blood samples, other body fluids, and tissues obtained during testing, operative procedures, or other standard medical practices for further research purposes. If applicable, statement of this option may be included within the informed consent document or may be provided as an addendum to the consent. A Model Consent Form for Use of Tissue for Research is available through a link in the DCP website.

Prior to study initiation, the informed consent document must be reviewed and approved by NCI, DCP, the Consortium Lead Organization, and the IRB at each Organization at which the protocol will be implemented. Any subsequent changes to the informed consent must be approved by NCI, DCP, the Consortium Lead Organization's IRB, and then submitted to each organization's IRB for approval prior to initiation.

14.5 Submission of Regulatory Documents

All regulatory documents are collected by the Consortia Lead Organization and reviewed for completeness and accuracy. Once the Consortia Lead Organization has received complete and

accurate documents from a participating organization, the Consortium Lead Organization will forward the regulatory documents to DCP's Regulatory Contractor:

Paper Document/CD-ROM Submissions:
Regulatory Affairs Department
CCS Associates, Inc.
2001 Gateway Place, Suite 350 West
San Jose, CA 95110
Phone: 650-691-4400
Fax: 650-691-4410

E-mail Submissions:
regulatory@ccsainc.com

Regulatory documents that do not require an original signature may be sent electronically to the Consortium Lead Organization for review, which will then be electronically forwarded to DCP's Regulatory Contractor.

14.6 Other

This trial will be conducted in compliance with the protocol, Good Clinical Practice (GCP), and the applicable regulatory requirements.

15. FINANCING, EXPENSES, AND/OR INSURANCE

Study subjects will be provided with a compensation as follows:

- $200 for each liver biopsy (at Screen 2 and at Visit 3)
- $75 for each clinic visit, for transportation, parking, childcare, etc. (Screen 1, Screen 2, Visit 1, Visit 2, Visit 3, and Visit 4).
- $25 for each weekly/bi-weekly blood draw between visits (max of 14). Participants will be provided $25 for the blood draw even if it is performed at an outside clinic.

The maximum total provided to each participant, depending on the requirements of their participation, is $1,200.

REFERENCES

1. Torre, L.A., et al., Global cancer statistics, 2012. CA Cancer J Clin, 2015. 65(2): p. 87-108.

2. El-Serag, H.B.; Davila, J.A.; Petersen, N.J.; McGlynn, K.A. The continuing increase in the incidence of hepatocellular carcinoma in the United States: An update. Ann. Intern. Med. 2003, 139, 817–823.

3. Llovet, J.M.; Burroughs, A.; Bruix, J. Hepatocellular carcinoma. Lancet 2003, 362, 1907–1917.

4. Trépo C, Chan HL, Lok A. Hepatitis B virus infection. Lancet. 2014;384:2053–2063.

5. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis. 2005;5:558–567.

6. Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. Gastroenterology. 2011;140:124–131.

7. Wong VW, Chu WC, Wong GL, Chan RS, Chim AM, Ong A, et al. Prevalence of non-alcoholic fatty liver disease and advanced fibrosis in Hong Kong Chinese: a population study using protonmagnetic resonance spectroscopy and transient elastography. Gut. 2012;61:409–415.

8. Kwok R, Choi KC, Wong GL, Zhang Y, Chan HL, Luk AO, et al. Screening diabetic patients for non-alcoholic fatty liver disease with controlled attenuation parameter and liver stiffness measurements: a prospective cohort study. Gut. 2015 pii: gutjnl-2015-309265.

9. O'Shea RS, Dasarathy S, McCullough AJ. Practice Guideline Committee of the American Association for the Study of Liver Diseases; Practice Parameters Committee of the American College of Gastroenterology. Alcoholic liver disease. Hepatology. 2010;51:307–328.

10. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380:2095–2128.

11. Lin DY, Sheen IS, Chiu CT, Lin SM, Kuo YC, Liaw YF. Ultrasonographic changes of early liver cirrhosis in chronic hepatitis B: a longitudinal study. J Clin Ultrasound. 1993;21:303–308.

12. European Association for the Study of the Liver; Asociacion Latinoamericana para el Estudio del Higado. EASL-ALEH Clinical Practice Guidelines: Non-invasive tests for evaluation of liver disease severity and prognosis. J Hepatol. 2015;63:237–264.

13. D'Amico G, Garcia-Tsao G, Cales P, Escorsell A, Nevens F, Cestari R, et al. Diagnosis of portal hypertension: how and when. In: DeFranchis R, editor. Portal hypertension III. Proceedings of the third Baveno international consensus workshop on definitions, methodology and therapeutic strategies. Hoboken, NJ: Blackwell Science; 2001. p. 36–64.

14. Garcia-Tsao G, Friedman S, Iredale J, Pinzani M. Now there are many stages where before there was one: in search of pathophysiological classification of cirrhosis. Hepatology. 2010;51: 1445–9.

15. Bruix, J.; Sherman, M. Management of hepatocellular carcinoma: An update. Hepatology 2011, 53, 1020–1022.

19. Bartsch H, Nair J (2006) Chronic inflammation and oxidative stress in the genesis and perpetuation of cancer: role of lipid peroxidation, DNA damage, and repair. Langenbecks Arch Surg 391(5):499-510.

20. Pan J, Chung FL (2002) Formation of cyclic deoxyguanosine adducts from ω-3 and ω-6 polyunsaturated fatty acids under oxidative conditions. Chem Res Toxicol 15(3):367-372.

21. Nair U, Bartsch H, Nair J (2007) Lipid peroxidation-induced DNA damage in cancer-prone inflammatory diseases: a review of published adduct types and levels in humans. Free Radic Biol Med 43(8):1109-1120.

22. Blair IA (2008) DNA adducts with lipid peroxidation products. J Biol Chem 283(23):15545-15549.

23. Marnett LJ (2000) Oxyradicals and DNA damage. Carcinogenesis 21(3):361-370.

24. Marra M, et al. (2011) Molecular targets and oxidative stress biomarkers in hepatocellular carcinoma: an overview. J Transl Med 9:171.

25. Bartsch H, Nair J (2004) Oxidative stress and lipid peroxidation-derived DNA-lesions in inflammation driven carcinogenesis. Cancer Detect Prev 28(6):385-391.

26. Nath RG, Chung FL (1994) Detection of exocyclic 1,N2-propanodeoxyguanosine adducts as common DNA lesions in rodents and humans. Proc Natl Acad Sci U S A 91(16):7491-7495.

27. Yang IY, et al. (2002) Mutagenesis by acrolein-derived propanodeoxyguanosine adducts in human cells. Biochemistry 41(46):13826-13832.

28. Minko IG, et al. (2003) Translesion synthesis past acrolein-derived DNA adduct, gamma -hydroxypropanodeoxyguanosine, by yeast and human DNA polymerase eta. J Biol Chem 278(2):784-790.

29. Wang HT, Zhang S, Hu Y, Tang MS (2009) Mutagenicity and sequence specificity of acrolein-DNA adducts. Chem Res Toxicol 22(3):511-517.

30. Kanuri M, et al. (2002) Error prone translesion synthesis past gamma-hydroxypropano deoxyguanosine, the primary acrolein-derived adduct in mammalian cells. J Biol Chem 277(21):18257-18265.

31. Feng Z, Hu W, Hu Y, Tang MS (2006) Acrolein is a major cigarette-related lung cancer agent: preferential binding at p53 mutational hotspots and inhibition of DNA repair. Proc Natl Acad Sci U S A 103(42):15404-15409.

32. Liu RH (2004) Potential synergy of phytochemicals in cancer prevention: mechanism of action. The J Nutr 134(12 Suppl):3479S-3485S.

33. Lee JE, Chan AT (2011) Fruit, vegetables, and folate: cultivating the evidence for cancer prevention. Gastroenterology 141(1):16-20.

34. Lippman SM, et al. (2009) Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). Jama 301(1):39-51.

35. Jungst C, et al. (2004) Oxidative damage is increased in human liver tissue adjacent to hepatocellular carcinoma. Hepatology 39(6):1663-1672.

36. Maki A, et al. (2007) Predictive power of biomarkers of oxidative stress and inflammation in patients with hepatitis C virus-associated hepatocellular carcinoma. Ann Surg Oncol 14(3):1182-1190.

37. Marquardt JU, Andersen JB, Thorgeirsson SS (2015) Functional and genetic deconstruction of the cellular origin in liver cancer. Nat Rev Cancer 15(11):653-667.

38. Yang JD, Roberts LR (2010) Hepatocellular carcinoma: a global view. Nat Rev Gastroenterol Hepatol 7(8):448-458.

39. Starley BQ, Calcagno CJ, Harrison SA (2010) Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection. Hepatology 51(5):1820-1832.

40. Lee HW, et al. (2014) Acrolein- and 4-Aminobiphenyl-DNA adducts in human bladder mucosa and tumor tissue and their mutagenicity in human urothelial cells. Oncotarget 5(11):3526-3540.

41. Ishikawa T, et al. (2004) DNA repair and cancer: lessons from mutant mouse models. Cancer Sci 95(2):112-117.

42. Guichard C, et al. (2012) Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. Nat Genet 44(6):694-698.

43 Peraino C, Fry RJ, Staffeldt E (1973) Brief communication: Enhancement of spontaneous hepatic tumorigenesis in C3H mice by dietary phenobarbital. J Natl Cancer Inst 51(4):1349-1350.

44 Verna L, Whysner J, Williams GM (1996) N-nitrosodiethylamine mechanistic data and risk assessment: bioactivation, DNA-adduct formation, mutagenicity, and tumor initiation. Pharmacol Ther 71(1-2):57-81.

45. Thirunavukkarasu C, Sakthisekaran D (2001) Effect of selenium on N-nitrosodiethylamine-induced multistage hepatocarcinogenesis with reference to lipid peroxidation and enzymic antioxidants. Cell Biochem Func 19(1):27-35.

46. Fu Y, et al. (2014) In vivo detection of a novel endogenous etheno-DNA adduct derived from arachidonic acid and the effects of antioxidants on its formation. Free Radic Biol Med 73:12-20.

47. Hasegawa R, et al. (1995) Preventive effects of green tea against liver oxidative DNA damage and hepatotoxicity in rats treated with 2-nitropropane. Food Chem Toxicol 33(11):961-970.

48. Westerterp-Plantenga MS (2010) Green tea catechins, caffeine and body-weight regulation. Physiol Behav 100(1):42-46.

49. Pan J, et al. (2012) Detection of acrolein-derived cyclic DNA adducts in human cells by monoclonal antibodies. Chem Res Toxicol 25(12):2788-2795.

50. Chow HH1, Cai Y, Alberts DS, Hakim I, Dorr R, Shahi F, Crowell JA, Yang CS, Hara Y. Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and polyphenon E. Cancer Epidemiol Biomarkers Prev. 2001 Jan;10(1):53-8.

51. Chow HH1, Hakim IA, Vining DR, Crowell JA, Ranger-Moore J, Chew WM, Celaya CA, Rodney SR, Hara Y, Alberts DS. Effects of dosing condition on the oral bioavailability of green

tea catechins after single-dose administration of Polyphenon E in healthy individuals. Clin Cancer Res. 2005 Jun 15;11(12):4627-33.

52. Chow HH1, Cai Y, Hakim IA, Crowell JA, Shahi F, Brooks CA, Dorr RT, Hara Y, Alberts DS. Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals Clin Cancer Res. 2003 Aug 15;9(9):3312-9.

53. Foster DR1, Sowinski KM, Chow HH, Overholser BR. Limited sampling strategies to estimate exposure to the green tea polyphenol, epigallocatechin gallate, in fasting and fed conditions. Ther Drug Monit. 2007 Dec;29(6):835-42.

54. Moura FA, de Andrade KQ, dos Santos JC, Goulart MO.(2015) Lipoic Acid: its antioxidant and anti-inflammatory role and clinical applications. Curr Top Med Chem. 2015;15(5):458-83.

55. Shanafelt TD1, Call TG, Zent CS, LaPlant B, Bowen DA, Roos M, Secreto CR, Ghosh AK, Kabat BF, Lee MJ, Yang CS, Jelinek DF, Erlichman C, Kay NE. Phase I trial of daily oral Polyphenon E in patients with asymptomatic Rai stage 0 to II chronic lymphocytic leukemia. J Clin Oncol. 2009 Aug 10;27(23):3808-14. doi: 10.1200/JCO.2008.21.1284. Epub 2009 May 26.

56. Shanafelt TD1, Call TG, Zent CS, Leis JF, LaPlant B, Bowen DA, Roos M, Laumann K, Ghosh AK, Lesnick C, Lee MJ, Yang CS, Jelinek DF, Erlichman C, Kay NE. Phase 2 trial of daily, oral Polyphenon E in patients with asymptomatic, Rai stage 0 to II chronic lymphocytic leukemia. Cancer. 2013 Jan 15;119(2):363-70. doi: 10.1002/cncr.27719. Epub 2012 Jul 3.

57. Isomura T, Suzuki S, Origasa H, Hosono A, Suzuki M, Sawada T, Terao S, Muto Y, Koga T.Liver-related safety assessment of green tea extracts in humans: a systematic review of randomized controlled trials.(2016) Eur J Clin Nutr. 2016 Nov;70(11):1221-1229. doi: 10.1038/ejcn.2016.78. Epub 2016 May 18.

58. Wang D, Wang Y, Wan X, Yang CS, Zhang J. (2015) Green tea polyphenol (-)-epigallocatechin-3-gallate triggered hepatotoxicity in mice: responses of major antioxidant enzymes and the Nrf2 rescue pathway. Toxicol Appl Pharmacol. 2015 Feb 15;283(1):65-74. doi: 10.1016/j.taap.2014.12.018. Epub 2015 Jan 10.

59. Ali Pezeshki, Sara Safi, Awat Feizi, Gholamreza Askari, and Fatemeh Karami. The Effect of Green Tea Extract Supplementation on Liver Enzymes in Patients with Nonalcoholic Fatty Liver Disease. Int J Prev Med. 2016; 7: 28.

60. Ryuichiro Sakata,Toru Nakamura, Takuji Torimura, Takato Ueno, Michio Sata Green tea with high-density catechins improves liver function and fat infiltration in non-alcoholic fatty liver disease (NAFLD) patients: A double-blind placebo-controlled study. Int J Mol Med. 2013 Nov;32(5):989-94. doi: 10.3892/ijmm.2013.1503. Epub 2013 Sep 20.

61. K. D. Crew, K. A. Ho, P. Brown, H. Greenlee1, T. B. Bevers, B. Arun, N. Sneige, C. Hudis3, H. L. McArthur, J. Chang, M. Rimawi, T. L. Cornelison, J. Cardelli, R. M. Santella1, A. Wang1, S. M. Lippman8, and D. L. Hershman. Effects of a green tea extract, Polyphenon E, on systemic biomarkers of growth factor signalling in women with hormone receptor-negative breast cancer. J Hum Nutr Diet. 2015 June ; 28(3): 272–282. doi:10.1111/jhn.12229.

62 Nagi B. Kumar, Julio Pow-Sang, Kathleen M. Egan, Philippe E. Spiess, Shohreh Dickinson, Raoul Salup, Mohamed Helal, Jerry McLarty, Christopher R. Williams, Fred Schreiber, Howard L. Parnes, Said Sebti, Aslam Kazi, Loveleen Kang, Gwen Quinn, Tiffany Smith, Binglin Yue1,

Karen Diaz, Ganna Chornokur, Theresa Crocker, and Michael J. Schell. Randomized, Placebo-Controlled Trial of Green Tea Catechins for Prostate Cancer Prevention. Cancer Prev Res (Phila). 2015 October ; 8(10): 879–887. doi:10.1158/1940-6207.CAPR-14-0324.

63. Jesus Lovera, MD, Alexander Ramos, MS, Deidre Devier, PhD, Virginia Garrison, RN, Blake Kovner, BS, Tara Reza, BS, Dennis Koop, PhD, William Rooney, PhD, Anne Foundas, MD, and Dennis Bourdette, M. Polyphenon E, Non-futile at Neuroprotection in Multiple Sclerosis but Unpredictably Hepatotoxic: Phase I Single Group and Phase II Randomized Placebo-Controlled Studies. J Neurol Sci. 2015 November 15; 358(0): 46–52. doi:10.1016/j.jns.2015.08.006.

64. Chow H-HS, Cai Y, Hakim IA, Crowell JA, Shahi F, Brooks CA, Dorr RT, Hara Y, Alberts DS. Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals. Clin Cancer Res 2003;9:3312–19.

65. Chow H-H, Hakim IA, Vining DR, Crowell JA, Ranger-Moore J, Chew WM, Celaya CA, Rodney SR, Hara Y, Alberts DS. Effects of dosing condition on the oral bioavailability of green tea catechins after single-dose administration of Polyphenon E in healthy individuals. Clin Cancer Res 2005;11:4627–33.

66. Chow H-H, Hakim IA, Vining DR, Crowell JA, Cordova CA, Chew WM, Xu M-J, Hsu CH, Ranger-Moore J, Alberts DS. Effects of repeated green tea catechin administration on human cytochrome P450 activity. Cancer Epidemiol Biomarkers Prev 2006;15:2473–76.

67. Bonkovsky HL. Hepatotoxicity associated with supplements containing Chinese green tea (Camellia sinensis). Ann Intern Med 2006;144:68–71.

68. Javaid A, Bonkovsky HL. Hepatotoxicity due to extracts of Chinese green tea (Camellia sinensis): A growing concern. J Hepatol 2006;45:334–36.

69. Sarma DN, Barrett ML, Chavez ML, Gardiner P, Ko R, Mahady GB, Marles RJ, Pellicore LS, Giancaspro GI, Low Dog T. Safety of green tea extracts: A systematic review by the US Pharmacopeia. Drug Saf 2008;31:469–84.

70. EASL-ALEH Clinical Practice Guidelines: Non-Invasive Tests for Evaluation of Liver Disease Severity and Prognosis. Journal of Hepatology 2015;63:237–264.

71. Rockey DC, Caldwell SH, Goodman ZD, Nelson RC, Smith AD. Liver Biopsy. Hepatology; 2008:49:1017–1044.

Consent Form Version date: 03/06/2023
Protocol Version and date: v4.13 03/06/2023

Consent Form

Study Title for Study Participants: Polyphenon E® to prevent liver cancer in people with liver cirrhosis.

Official Study Title for Internet Search on http://www.ClinicalTrials.gov: NWU2016-08-02 A phase I single-arm, multicenter pilot study aimed at validating γ-OHPdG as a biomarker and testing the effects of Polyphenon E on its levels in patients with cirrhosis.

Introduction

This is a clinical trial, a type of research study. Your study doctor will explain the clinical trial to you. Clinical trials include only people who choose to take part in the research. Please take your time to make your decision about volunteering. You may discuss your decision with your friends and family. You can also discuss this study with your health care team. If you have any questions, you can ask your study doctor for more of an explanation. You should only agree to participate in this study when you are comfortable enough with the information so that you can make an informed decision about joining.

What is the usual approach to my Liver Cirrhosis?

You are being asked to take part in this study because you have liver cirrhosis and are at increased risk for liver cancer. People who have liver cirrhosis and choose not to participate in a study are usually followed closely by their doctor to manage their cirrhosis and to find cancer if it occurs.

What are my other choices if I do not take part in this study?

If you decide not to take part in this study, you have other choices. For example:

- you may choose to have the usual approach described above,
- you may choose to take part in a different study, if one is available,
- or you may choose to do nothing.

Why is this study being done?

We hope to determine how well cirrhotic participants tolerate different doses of green tea extract (Polyphenon E). We also hope to determine if green tea extract may decrease the level of a certain molecule called γ-OHPdG ("gamma"-OHPdG), and whether high levels of that molecule are associated with a higher risk of liver cancer.

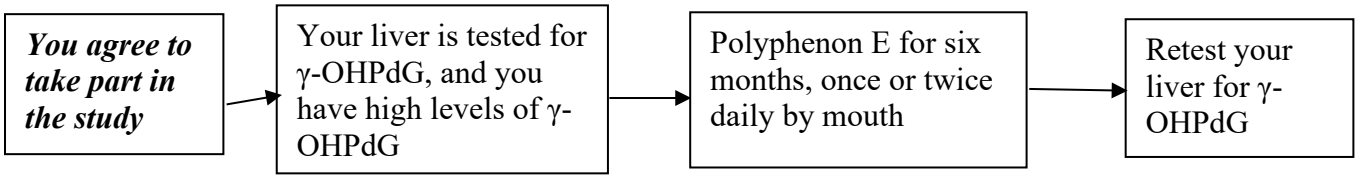
What are the study groups?

If you chose to participate, your liver will be tested to see if it is making a molecule called γ-OHPdG. If your liver shows high levels of γ-OHPdG, you will receive the study drug

Polyphenon E. Different doses of Polyphenon E will be given to several study participants. What dose you receive will depend on when you enroll in the study.

Dose escalation part (the first five groups): In the first part of the study, several participants will receive Polyphenon E at a low dose. If the study drug does not cause bothersome side effects, it will be given to the second group of study participants at a higher dose. If the low dose causes bothersome side effects in one or more people in the first group, it will be given to additional study participants at the same dose to get a better idea of how often the study drug is bothersome. In this way, we hope to determine the safest/best tolerated dose of Polyphenon E. Once this dose of Polyphenon E is found, then the next part of the study will begin.

Dose expansion part (the sixth group): Twenty-four participants will receive the safest/best tolerated dose of the study drug to learn more about how the drug affects the liver.



How long will I be in this study?

You will receive the study drugs for *6 months*. Even if you do not finish the study, your doctor will continue to watch you for side effects and follow your condition for *2 months*.

What extra tests and procedures will I have if I take part in this study?

Most of the exams, tests, and procedures you will have are part of the usual approach for your condition. However, there are some extra tests that you will need to have if you take part in this study.

Before you begin the study, you will need to have the following extra tests to find out if you can be in the study:
Screen 1 Visit

- FibroScan scan of your abdomen (if not already done within the past 3 months) as part of standard medical care; ultrasound, CT or MRI may be performed (if not already done within the past 3 months) as part of standard medical care
- Blood tests to make sure you are healthy
- Pregnancy test if you are female and able to have children

Screen 2 Visit
A biopsy (small pieces of liver tissue) is obtained by inserting a needle into the liver for a fraction of a second. This research biopsy is done in a similar way to biopsies are done for diagnosis. It is done in the hospital, and you will be sent home within two to three hours. This biopsy will be taken at the Screen 2 Visit.

If you had a liver biopsy in the past year, let the study team know. If tissue from your liver biopsy in the past year was kept at the hospital, and can be used for the study, the Screen 2 biopsy is optional for you.

For participants who do not have tissue from a biopsy in the last year that was kept at the hospital, this biopsy is required in order for you to take part in this study. You will sign a separate consent form before the biopsy is taken. This will be a standard surgical consent form from the institution where the biopsy procedure takes place.

We will test the biopsy sample for levels of a substance called γ-OHPdG, and you will only be treated on the study if your liver shows high levels of γ-OHPdG.

A urine sample will also be collected to see the levels of γ-OHPdG in order to compare the levels found in your liver biopsy.

The study coordinator will call you within around four weeks of Screen 1 and/or Screen 2 to let you know if you are eligible to continue in the study.

If the liver biopsy shows that you have high levels of γ-OHPdG, and you choose to take part in the study, then you will need the following extra tests. They are not part of the usual approach for your liver cirrhosis.

During the study:
Visit 1 (Week 1 Day 1, the first day you start taking the study drug)

- Physical examination and vital signs (for example, heart rate and blood pressure tests), and answering some questions about your medical history, other medications you are taking, and how the study drug is affecting you.
- Answer questions about your tobacco and alcohol use
    - Researchers want to see if tobacco and alcohol use affects the side effects people might get while on this study, or if tobacco and alcohol use modifies the effects of the study agent
- Blood tests to make sure you have good organ function.
- Blood tests to (1) measure the levels of γ-OHPdG in your blood.
- **Optional (Georgetown University ONLY)**: Extra blood and urine tests used to study the way the body absorbs, distributes, and gets rid of a drug. This is referred to as a pharmacokinetics pharmacokinetics (PK) study.

  A PK study is when samples (such as blood and urine) are collected before and after taking a drug to see how your body absorbs, distributes, breaks down, and eliminates the drug.

  If you are one of the first six people who agree to provide extra blood and urine for the PK study: before you take your first dose of the study drug, two urine samples and four extra tubes of blood will be drawn to make sure you are healthy. You will then stay at the clinic for 9 hours after your first dose of the study drug to have three additional blood draws and three separate urine tests to see how your body processes the study

drug. The study coordinator will let you know if you are one of the first six people who can participate in this.

Between Visits 1 and 2

- Every week or every other week you will have one blood draw to make sure you are healthy and have good organ function. You may have it done at one of the scheduled visits, or have it done in LabCorp, or Quest lab, or your doctor's office when you do not have a scheduled visit with your liver doctor.

Visit 2 (Week 13, 12 weeks after you start taking the study drug)

- Physical examination and vital signs, and answering some questions about your medical history, other medications you are taking, and how the study drug is affecting you.
- Blood tests to make sure you are healthy and have good organ function.
- Urine test to measure the amount of γ-OHPdG in your body
- Blood tests to (1) measure levels of γ-OHPdG in your blood.

Between Visits 2 and 3

- Every week or every other week you will have one blood draw to make sure you have a good organ function. You may have it done at one of the scheduled visits, or have it done in LabCorp, or Quest lab, or your doctor's office when you do not have a scheduled visit with your liver doctor.

Visit 3 (Week 24, at the end of treatment with the study drug)

- Physical examination and vital signs, and answering some questions about your medical history, other medications you are taking, and how the study drug is affecting you.
- Answer questions about your tobacco and alcohol use
    - Researchers want to see if tobacco and alcohol use affects the side effects people might get while on this study, or if tobacco and alcohol use modifies the effects of the study agent
- Blood tests to make sure you are healthy and have good organ function.
- Urine test to measure the amount of γ-OHPdG in your body
- Blood tests to (1) measure levels of γ-OHPdG in your blood.
- Ultrasound, CT or MRI scan, and FibroScan of your abdomen. These tests may be done at the same visit, or at a separate visit within 3 weeks before or after your last day on the study drug.
- Liver biopsy. The liver biopsy during Visit 3 is required for all participants on the study.

Visit 4 (Week 28, 4 weeks after you stop taking the study drug)

- Physical examination and vital signs, and answering some questions about your medical history, other medications you are taking, and how the study drug is affecting you.
- Blood tests to make sure you are healthy and have good organ function.

How should I take this medication?

The Polyphenon E/EGCG capsules are recommended to be taken with a cup of water after a light meal without milk or juice during the meal. Patient should not make up the missing dose the next day. Patient should not make up vomited doses. The missing dose or vomited dose should be reported in the medication diary.

What possible risks can I expect from taking part in this study?

If you choose to take part in this study, there is a risk that you may:

1) Lose time at work or home and spend more time in the hospital or doctor's office than usual.
2) Be asked sensitive or private questions which you normally do not discuss, for example about your tobacco and alcohol use.
3) There is a risk someone could get access to the personal information in your medical records or other information researchers have kept about you. Someone might be able to trace this information back to you. The researchers believe the chance that someone will identify you is very small, but the risk may change in the future as people come up with new ways of tracing information. In some cases, this information could be used to make it harder for you to get or keep a job.

The Polyphenon E used in this study may affect how different parts of your body work, such as your liver, kidneys, heart, and blood. The study doctor will be testing your blood and will let you know if changes occur that may affect your health.

Liver damage has been reported following use of green tea-containing extracts. In at least one case, liver failure occurred. There is no available information on the safety of administering Polyphenon E to participants with underlying cirrhosis/ liver disease, but the risk of that it may cause worsening liver damage or liver failure could be higher than the risk of administering Polyphenon E to participants with no underlying liver disease/ cirrhosis.

We will be monitoring your liver tests closely during this study.

It is not known if Polyphenon E/green tea extract consumption will directly benefit your liver disease. It is also not known whether six months of Polyphenon E will decrease the risk of liver cancer. The goal of the study is to test if Polyphenon E will decrease the biomarker that correlates with liver cancer development.

You may or may not have side effects. Everyone taking part in this study will be watched carefully for any side effects.

Here are important points about side effects:

1) The study doctors do not know who will or will not have side effects.
2) Some side effects may go away soon, some may last a long time, or some may never go away.
3) Some side effects may interfere with your ability to have children.
4) Some side effects may be serious and may even result in death.

Here are important points about how you and the study doctor can make side effects less of a problem:

5) Tell the study doctor if you notice or feel anything different so they can see if you are having a side effect.
6) The study doctor may be able to treat some side effects.

The tables below show the most common side effects that we know about Polyphenon E, some of which may be serious. There might be other side effects that we do not yet know about. If important new side effects are found, the study doctor will discuss these with you.

Possible Side Effects of Polyphenon E

COMMON, SOME MAY BE SERIOUS In 100 people receiving Polyphenon E, more than 20 may have:
• Headache • Heartburn • Abnormal blood tests (related to how well liver is working) • Nausea

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving, from 4 to 20 may have:
• Abnormal blood tests (related to how well liver, kidney, and pancreas are working) • Abnormal heartbeat • Abnormal laboratory test (increased protein in the urine, sugar present in the urine) • Abnormal menstrual period • Abdominal discomfort • Bleeding of the rectum • Bloating • Burping • Changes in taste • Chest pain • Constipation • Diarrhea • Difficulty sleeping • Discomfort from light • Dizziness • Flushing • Gas • Inflammation (swelling and redness) of the stomach lining • Itching • Low blood level of hemoglobin, a protein that carries oxygen in the body • Low level of blood bicarbonate • Nose bleed • Pain (pain in the belly, pain in the mouth, pain in the muscle, pain in the leg/arm) • Rash • Ringing in the ear • Swelling and redness of skin • Stuffy nose

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving, from 4 to 20 may have:
• Swelling of the eyelid • Temporary lowering of the number of white blood cell count, which can lead to infection • Tiredness • Vomiting • Weight increased

RARE, SOME MAY BE SERIOUS In 100 people receiving Polyphenon E, 3 or fewer may have:
• Loss of appetite • Widening of blood vessels (vasodilation)

Reproductive risks: You should not get pregnant, breastfeed, or father a baby while in this study. The Polyphenon E used in this study could be very damaging to an unborn baby. Contraception must be used prior to study entry and throughout the study. Check with the study doctor about what types of birth control, or pregnancy prevention, to use while in this study.

Risk of the blood tests: Bruising, soreness, or rarely, infection may occur as a result of the needle sticks to obtain blood from your vein.

Risks of liver biopsy: The primary risk of liver biopsy is bleeding from the site of needle entry into the liver, although this occurs in less than one per cent of participants. When it occurs, it usually stops on its own and does not lead to any other problems. Other rare complications include infection and the puncture of other organs, such as the kidney, lung or colon. A liver biopsy procedure that damages the gallbladder by mistake may lead to leakage of bile into the abdominal cavity, causing peritonitis. The risk of death from liver biopsy is extremely low, about 1 in 5,000. Common side effects of a biopsy are a small amount of bleeding at the time of the procedure, pain at the biopsy site, which can be treated with regular pain medications, and bruising.

Risks of FibroScan: There are no known risks associated with a FibroScan examination.

What possible benefits can I expect from taking part in this study?

Participating in this study may or may not help your condition. This study may help us learn things that could help people in the future.

Can I stop taking part in this study?

Yes. You can decide to stop at any time. If you decide to stop for any reason, it is important to let the study doctor know as soon as possible so you can stop safely. Your study doctor will tell you how to stop taking the study drug and how to return the study drug you haven't taken. There are no consequences to stopping the study. If you stop, you can decide whether or not to let the study doctor continue to provide your medical information to the organization running the study.

For the tobacco and alcohol use questions, you can decide to not answer some or all of the questions. Your decision will not affect whether you can participate in the study, and it will not affect your relationship with your doctor or the study staff.

The study doctor will tell you about any new information or changes in the study that could affect your health or your willingness to continue in the study.

The study doctor may take you out of the study:

a) If your health changes, for example, worsening liver function from cirrhosis, or development of liver cancer.
b) If the study is no longer in your best interest.
c) If new information becomes available, indicating there is a new treatment to prevent liver cancer, or green tea extract is harmful to your health.
d) If you do not follow the study rules.
e) If the study is stopped early for any reason by the sponsor, IRB or FDA.

What are my rights in this study?

Taking part in this study is your choice. No matter what decision you make, and even if your decision changes, there will be no penalty to you. You will not lose medical care or any legal rights.

For questions about your rights while in this study, call the National Cancer Institute Central Institutional Review Board at 1-888-657-3711.

What are the costs of taking part in this study?

The Polyphenon E will be supplied at no charge while you take part in this study. The cost of study-specific biopsies and exams, blood tests, and any other procedures will be paid for by the study.

Some costs associated with your care may be considered standard of care, and will be billed to you or your insurance company. You will have to pay for any costs (including deductibles and co-payments) not covered by your health insurer.

Before you decide to be in the study, you should check with your health plan or insurance company to find out exactly what they will pay for.

You will be provided $200 for each liver biopsy you have for this study (at Screen 2 and at Visit 3)). You will be provided $75 for each clinic visit (Screen 1, Screen 2, Visit 1, Visit 2, Visit 3, and Visit 4). You will be provided $25 for each between-visit blood draw you need for this study

(up to 14). Depending on what activities you participate in, the maximum total you may be provided is $1,200.

What happens if I am injured or hurt because I took part in this study?

If you feel you have been injured or hurt as a result of taking part in the study, it is important that you tell the study doctor immediately. You will get medical treatment if you are injured or hurt as a result of taking part in this study.

The study sponsors will not offer to pay for medical treatment for injury. Your insurance company may not be willing to pay for study-related injury. If you have no insurance coverage, you would be responsible for any costs. Even though you are in a study, you keep all of your legal rights to receive payment for injury caused by medical errors.

Who will see my medical information?

Your privacy is very important to us and we will make every effort to protect it. Your information may be given out if required by law. For example, certain states require doctors to report to health boards if they find a disease like tuberculosis. The study doctors have a privacy permit to help protect your records if there is a court case. However, we will do our best to make sure that any information that is released will not be able to identify you. Some of your health information, and/or information about your specimen, from this study will be kept in a central database for research. Your name or contact information will not be put in the database.

There are organizations that may inspect your records. These organizations are required to make sure your information is kept private. Some of these organizations are:

1) The study sponsor and any drug company supporting the study
2) The Institutional Review Board, IRB, is a group of people who review the research with the goal of protecting the people who take part in the study.
3) The Food and Drug Administration and the National Cancer Institute in the US.
4) The National Cancer Institute will obtain information for this clinical trial under data collection authority Title 42 U.S.C. 285.

Where can I get more information?

The National Cancer Institute will obtain information from this clinical trial under data collection authority Title 42 U.S.C. 285.

You may visit the NCI website at http://cancer.gov/ for more information about studies or general information about cancer. You may also call the NCI Cancer Information Service to get the same information at: 1-800-4-CANCER (1-800-422-6237).

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by US law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

Who can answer my questions about this study?

You can talk to the study doctor about any questions or concerns you have about this study or to report side effects or injuries.

If you are a patient of Medstar Georgetown University Hospital, contact the study doctor Dr. Coleman Smith at 202-444-3700. If you need to contact study staff for emergencies or outside normal hours, you can call 202-444-2000 and ask for the hepatologist on call.

If you are a patient of Medstar Washington Hospital Center, contact the study doctor Dr. Arul Thomas at 202-444-3700. If you need to contact study staff for emergencies or outside normal hours, you can call 202-444-2000 and ask for the hepatologist on call.

If you are a patient of the University of Puerto Rico, contact the study doctor Dr. Marcia Cruz, at 787-772-8300. If you need to contact study staff for emergencies or outside normal hours, you can call 787-772-8300.

This section is about optional studies you can choose to take part in.

This part of the consent form is about optional studies that you can choose to take part in. You will not get health benefits from any of these studies. The researchers leading this optional study hope the results will help other people with cancer in the future.

The results will not be added to your medical records, and you or your study doctor may not know the results. You will not be billed for these optional studies.

You can still take part in the main study even if you say 'no' to any or all of these studies. If you sign up for but cannot complete any of the studies for any reason, you can still take part in the main study.

Circle your choice of "yes" or "no" for each of the following studies.

Optional Sample Collections for Laboratory Studies and/or Biobanking for Possible Future Studies

Researchers are trying to learn more about cancer, diabetes, and other health problems. Much of this research is done using samples from your biopsies, blood, urine, or other fluids. Through these studies, researchers hope to find new ways to prevent, detect, treat, or cure health problems.

Some of these studies may be about genes. Genes carry information about features that are found in you and in people who are related to you. Researchers are interested in the way that genes affect how your body responds to treatment.

The researchers would like to ask your permission to store and use your leftover samples and health information obtained during your participation in this study for future medical research. Storing samples for future studies is called "biobanking". The Biobank is being run and supported by The National Cancer Institute.

The research that may be done is unknown at this time. Future research may include: 1) studies

to identify genes and/or biomarkers and proteins that influence a risk of cancer in people with liver disease; 2) studies to identify specific pathways and mechanisms that promote cancer. A biomarker is a biological molecule found in blood, other body fluids, or tissues that may be a sign of a condition or disease.

WHAT IS INVOLVED?

If you agree to take part, here is what will happen next:

1. If you are one of the first six people at Georgetown University who agree to provide extra blood and urine for the PK study: you will be asked to fast after midnight except for drinking water prior to coming into the clinic for Visit 1. Before you take your first dose of the study drug, two urine samples and four extra tubes of blood will be drawn to make sure you are healthy. You will then stay at the clinic for 9 hours after your first dose of the study drug to have three additional blood draws and three separate urine tests to see how your body processes the study drug. The additional blood tests only will be obtained from the first six participants who wish to participate in these additional tests.
2. You will need to have liver tissue tested for this study. If you have had a liver biopsy in the past year and tissue from that biopsy was stored at the hospital. , you can either:
    a) Give permission for the stored tissue to be used for the study, and also agree to have an additional liver biopsy at Screen 2, OR
    b) Give permission for the stored tissue to be used for the study, but decide NOT to have another liver biopsy at Screen 2, OR
    c) Don't give permission for the stored tissue to be used for the study, but instead agree to have a liver biopsy at Screen 2
3. Your leftover samples and some related information may be stored in the Biobank, along with samples and information from other people who take part. The samples will be stored at Georgetown University or the University of Puerto Rico until the end of the study, when they may be transferred to the National Institutes of Health. These are not additional samples that will be collected, but will consist of any material (tissue, urine, and blood samples) that remains after the tests described for this study have been conducted.
4. Qualified researchers can submit a request to use the materials stored in the Biobank. A research committee will review each request. There will also be an ethics review to ensure that the request is necessary and proper. Researchers will not be given your name or any other information that could directly identify you.
5. Neither you nor your study doctor will be notified if/when research is conducted using your samples.
6. Some of your genetic and health information may be placed in central databases that may be public, along with information from many other people. Information that could directly identify you will not be included.

WHAT ARE THE POSSIBLE RISKS?

1. The most common risks related to drawing blood from your arm are brief pain and possibly a bruise.
2. There is a risk that someone could get access to the personal information in your medical records or other information we have stored about you.
3. There is a risk that someone could trace the information in a central database back to you. Even without your name or other identifiers, your genetic information is unique to you.

The researchers believe the chance that someone will identify you is very small, but the risk may change in the future as people come up with new ways of tracing information.

There are laws against the misuse of genetic information, but they may not give full protection. New health information about inherited traits that might affect you or your blood relatives could be found during a study. The researchers believe the chance these things will happen is very small, but cannot promise that they will not occur.

A new Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. This law generally will protect you in the following ways:

- Health insurance companies and group health plans may not request your genetic information that we get from this research.
- Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility or premiums.

Employers with 15 or more employees may not use your genetic information that we get from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment. All health insurance companies and group health plans must follow this law by May 21, 2010. All employers with 15 or more employees must follow this law as of November 21, 2009.

Be aware that this new Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

HOW WILL INFORMATION ABOUT ME BE KEPT PRIVATE?

Your privacy is very important to the researchers and they will make every effort to protect it. Here are just a few of the steps they will take:

1. When your sample(s) is sent to the researchers, no information identifying you (such as your name or social security number) will be sent. Samples will be identified by a unique study code only. (Note to consent form authors: If investigators are receiving samples directly from sites without being coded, modify accordingly.)
2. The list that links the unique code to your name will be kept separate from your sample and health information. Any Biobank and *(insert name of clinical trials organization)* staff with access to the list must sign an agreement to keep your identity confidential.
3. Researchers to whom *(insert name of clinical trials organization)* sends your sample and information will not know who you are. They must also sign an agreement that they will not try to find out who you are.
4. Information that identifies you will not be given to anyone, unless required by law.
5. If research results are published, your name and other personal information will not be used.

WHAT ARE THE POSSIBLE BENEFITS?

You will not benefit from taking part. The researchers, using the samples from you and others, might make discoveries that could help people in the future.

ARE THERE ANY COSTS OR PAYMENTS?

There are no costs to you or your insurance. You will not be paid for taking part. If any of the research leads to new tests, drugs, or other commercial products, you will not share in any profits.

WHAT IF I CHANGE MY MIND?

If you decide you no longer want your samples to be used, you can call the study doctor, _____, *(insert name of study doctor for main trial)* at _____ *(insert telephone number of study doctor for main trial)* who will let the researchers know. Then, any sample that remains in the bank will no longer be used. Samples or related information that have already been given to or used by researchers will not be returned.

WHAT IF I HAVE MORE QUESTIONS?

If you have questions about the use of your samples for research, contact the study doctor, _____, *(insert name of study doctor for main trial)*, at _____ *(insert telephone number of study doctor for main trial)*.

Please circle your answer to show whether or not you would like to take part in each option:

SAMPLES AND INFORMATION FOR FUTURE RESEARCH STUDIES:

<u>If you are one of the first six participants at Georgetown University who agrees to participate in the PK study:</u>
Participate in the PK study to study how the body absorbs, distributes, breaks down, and eliminates the study drug (three additional blood draw and urine test on Visit 1 (week 1 day 1):

YES NO N/A

My samples and related information may be kept in the NCI Biobank for use in future health research. These are blood samples leftover from the main study.

YES NO

I agree that my study doctor, or their representative, may contact me or my physician to see if I wish to participate in other research in the future.

YES NO

The information from my tobacco and alcohol use questionnaires may be used in future health research.

YES NO

I provide permission to my study doctor, or their representatives, to contact me once the entire study has been completed for the purposes of sending study results when they

become available

YES NO

I have had a liver biopsy in the past year, and the tissue was stored at the hospital

YES NO

If YES:

I agree that the liver biopsy tissue stored at the hospital can be used for this study

YES NO N/A

If NO:

I agree to undergo an additional, optional liver biopsy at the Screen 2 Visit

YES NO N/A

This is the end of the section about optional studies.

My Signature Agreeing to Take Part in the Main Study

I have read this consent form or had it read to me. I have discussed it with the study doctor and my questions have been answered. I will be given a signed copy of this form. I agree to take part in the main study.

Participant's signature _____

Date of signature_____

Signature of person(s) conducting the informed consent discussion

_____

Date of signature_____

APPENDIX A
Performance Status Criteria

ECOG Performance Status Scale

Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

Karnofsky Performance Scale

Percent	Description
100	Normal, no complaints, no evidence of disease.
90	Able to carry on normal activity; minor signs or symptoms of disease.
80	Normal activity with effort; some signs or symptoms of disease.
70	Cares for self, unable to carry on normal activity or to do active work.
60	Requires occasional assistance, but is able to care for most of his/her needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization indicated. Death not imminent.
20	Very sick, hospitalization indicated. Death not imminent.
10	Moribund, fatal processes progressing rapidly.
0	Dead.

APPENDIX B

Instructions for pills

1. Store at room temperature
2. Keep canisters out of reach of children
3. If you can, take your pills at the same time. If you forget to take it at the same time, take it any time that you remember.
4. If you prefer to take the pills at another time (for example, when you take your other medications) that is OK, as long as you remember to take it daily.
5. Always take the pills after a light meal.
6. If you start any new medications while you are on study, please call your study coordinator. She will check with the study doctor and if needed, with your personal physician so that possible interactions can be managed safely.
7. Avoid fasting or taking vitamin C, fish oil , calcium, magnesium, or milk with the Polyphenon capsules. You should take your Polyphenon E/EGCG capsules with a cup of water after a light meal without milk or juice during the meal. You should not make up the missing dose the next day. You should not make up vomited doses. The missing dose or vomited dose should be reported in the medication diary.

APPENDIX C
Participant Diary

Participant ID_____

DAY ___	DAY ___	DAY ___	DAY ___	DAY ___	DAY ___	DAY ___
DATE: ___/____/____ TIME: _____ TIME: _____	DATE: ___/____/____ TIME: _____ TIME: _____	DATE: ___/____/____ TIME: _____ TIME: _____	DATE: ___/____/____ TIME: _____ TIME: _____	DATE: ___/____/____ TIME: _____ TIME: _____	DATE: ___/____/____ TIME: _____ TIME: _____	DATE: ___/____/____ TIME: _____ TIME: _____
Please list any new symptoms, medications, or reasons for missed doses:	Please list any new symptoms, medications, or reasons for missed doses:	Please list any new symptoms, medications, or reasons for missed doses:	Please list any new symptoms, medications, or reasons for missed doses:	Please list any new symptoms, medications, or reasons for missed doses:	Please list any new symptoms, medications, or reasons for missed doses:	Please list any new symptoms, medications, or reasons for missed doses:

Study Coordinator Signature: _____ Date: _____

APPENDIX D
Hepatotoxicity Assessment Algorithm

Baseline ALT or AST	Liver toxicities	Treatment and follow up plan
Normal	ALT or AST increases to ≥5x upper limit of normal AND no liver related symptoms (nausea, vomiting, right upper quadrant pain)	Repeat liver profile (AST, ALT, bilirubin (total, direct, and indirect) and INR) within 3 to 4 days. Email NCPC and the protocol PI within 48 hours of learning about the event (ncpc@northwestern.edu and arh29@georgetown.edu).
Elevated	ALT or AST increases to ≥3x baseline levels AND no liver related symptoms (nausea, vomiting, right upper quadrant pain)	Interrupt the study medication. Initiate potential DILI work-up for competing etiologies, repeat liver profile and INR within 48-72 hours. Study medication can be restarted only if a firm competing etiology is identified and liver tests return to baseline. Email NCPC and the protocol PI within 48 hours of learning about the event (ncpc@northwestern.edu and arh29@georgetown.edu).
Irrespective of baseline levels	ALT or AST increase to ≥3x baseline AND symptoms of clinical hepatitis (vomiting, nausea, right upper quadrant pain)	Interrupt the study medication. Initiate potential DILI work-up for competing etiologies, repeat liver profile and INR within 48-72 hours. Study medication can be restarted only if a firm competing etiology is identified and liver tests return to baseline. Email NCPC and the protocol PI within 48 hours of learning about the event (ncpc@northwestern.edu and arh29@georgetown.edu).
Irrespective of baseline levels	Elevations in direct bilirubin >1.5 x ULN, and if ALT or AST ≥3 x baseline but without liver related symptoms (vomiting, nausea, right upper quadrant pain)	Interrupt the study medication. Initiate potential DILI work-up for competing etiologies, repeat liver profile and INR within 48-72 hours. Study medication can be restarted only if a firm competing etiology is identified and liver tests return to baseline. Email NCPC and the protocol PI within 48 hours of learning about the event (ncpc@northwestern.edu and arh29@georgetown.edu).
Irrespective of baseline levels	With any elevation of direct bilirubin > ULN, regardless of ALT or AST levels, AND with indicators of immunological reaction (i.e., rash or >5% eosinophilia), or appearance of nausea, vomiting, right upper quadrant pain (symptoms consistent with clinical hepatitis)	Permanently discontinue study medication. Initiate potential DILI work-up for competing etiologies, repeat liver profile and INR within 48-72 hours, and place patient under "close observation" as defined in the DILI guidance. Email NCPC and the protocol PI within 48 hours of learning about the event (ncpc@northwestern.edu and arh29@georgetown.edu).

Criteria for taking patients off agent:

1) If close monitoring of a patient is not possible, or
2) Any elevation of direct bilirubin > ULN, regardless of ALT or AST levels, AND with indicators of immunological reaction (i.e., rash or >5% eosinophilia), or appearance of nausea, vomiting, right upper quadrant pain (symptoms consistent with clinical hepatitis)
3) Any development of recurrent hepatotoxicity (as outlined in this table) upon rechallenge.
4) Any hepatic decompensation such as
   - New onset ascites or worsening of ascites (requiring increase in drug therapy or requiring surgical intervention such as paracentesis).
   - Variceal bleeding documented by endoscopy
   - Spontaneous bacterial peritonitis documented by positive culture
   - Hepatic encephalopathy
   - Hepatorenal syndrome (Type 1 or 2)
   - Porto-pulmonary hypertension
   - Hepato-pulmonary hypertension
   - Any liver related event that leads to hospitalization or G4 event.

Email NCPC and the protocol PI within 48 hours of learning a participant needs to be taken off agent (ncpc@northwestern.edu and arh29@georgetown.edu).

If a participant meets any of these criteria, email NCPC and the protocol PI within 48 hours of learning about the event (ncpc@northwestern.edu and arh29@georgetown.edu).

1. If direct bilirubin remains in the normal range during the trial and only transaminase elevations are observed:
   - When the baseline ALT or AST are normal at baseline and increase to ≥5x upper limit of normal (ULN) during the trial **AND** the patient does not have liver related symptoms (nausea, vomiting, right upper quadrant pain) then
     - *Repeat liver profile (AST, ALT, Bilirubin (total, direct, and indirect)) and INR within 3 to 4 days. Monitor the patient as per "close observation" definition in the DILI guidance.*

   - When the ALT or AST are elevated at baseline and increase to ≥3x baseline during the trial **AND** the patient does not have liver related symptoms (nausea, vomiting, right upper quadrant pain) then
     - *Interrupt the study medication. Initiate potential DILI work-up for competing etiologies, repeat liver profile and INR within 48-72 hours, and place patient under "close observation" as defined in the DILI guidance. Study medication can be restarted only if a firm competing etiology is identified and liver tests return to baseline.*

   - When the ALT or AST increase to ≥3x baseline (irrespective of baseline levels) during the trial and are associated with symptoms of clinical hepatitis such as vomiting, nausea, right upper quadrant pain), then
     - *Interrupt the study medication. Initiate potential DILI work-up for competing etiologies, repeat liver profile and INR within 48-72 hours, and place patient under "close observation" as defined in the DILI guidance. Study medication can be restarted only if a firm competing etiology is identified and liver tests return to baseline.*

2. For patients with elevations in direct bilirubin > 1.5 x ULN, and if ALT or AST ≥3 x baseline but without liver related symptoms (vomiting, nausea, right upper quadrant pain) then
   - *Interrupt the study medicine. Initiate potential DILI work-up for competing etiologies, repeat liver profile and INR within 48-72 hours, and closely observe the patient. For "close observation" of patients, refer to the DILI guidance[1]. Study medication can be restarted only if a firm competing etiology is identified and the liver tests return to baseline.*

3. With any elevation of direct bilirubin > ULN, regardless of ALT or AST levels, and in the presence of indicators of immunological reaction (i.e., rash or >5% eosinophilia), or appearance of nausea, vomiting, right upper quadrant pain (symptoms consistent with clinical hepatitis) then
   - *Permanently discontinue study medication. Initiate potential DILI work-up for competing etiologies, repeat liver profile and INR within 48-72 hours, and place patient under "close observation" as defined in the DILI guidance.*

If a patient lives in a remote area, they can be tested locally and the results communicated to the investigator site promptly.

Close observation:

1. Interrupt the investigational agent administration
2. Repeat liver biochemistries and additional testing within 24-72 hrs

[1] (refer to the "Guidance for Industry : Drug induced liver injury (DILI): Premarketing clinical evaluation" ***http://www.fda.gov/downloads/Drugs/.../Guidances/UCM174090.pdf)***

3. Monitor patient twice or thrice a week until liver biochemistries (ALT, AST, alkaline phosphatase, bilirubin (total, direct, and indirect), and coagulation profile [INR]) resolve, stabilize or return to within baseline values
4. Monitor liver biochemistries once a week if abnormalities stabilize and the patient is asymptomatic
5. Obtain a detail history for symptoms assessment: appearance or worsening of clinical symptoms of hepatitis (fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash or eosinophilia). If a patient is symptomatic, the drug must be discontinued immediately and a potential DILI evaluation must be performed.
6. Obtain a more detailed history of symptoms and prior or concomitant diseases;
7. Obtain a history for concomitant medications, acetaminophen, dietary supplements, herbal remedies, other over the counter medications, recreational drug use, and special diets
8. If possible quantify the alcohol consumption to assess for alcoholic hepatitis
9. Obtain a history of exposure to environmental chemical agents.
10. If INR is also elevated, a trial of intravenous vitamin K administration may be considered, especially in cholestatic patients.

Follow-Up Procedures for patient(s) who meet *potential DILI evaluation criteria*:

1. Viral hepatitis serology including:
    a. Hepatitis A IgM antibody;
    b. Hepatitis B surface antigen and Hepatitis B Core Antibody (IgM);
    c. Hepatitis C RNA;
    d. Hepatitis E IgM antibody.
    e. Cytomegalovirus IgM antibody;
    f. Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing); however IgM antibodies must be sent out asap
    g. Blood sample for pharmacokinetic (PK) analysis, obtained within 12 hours of last dose. Record the date/time of the PK blood sample draw and the date/time of the last dose of study treatment prior to blood sample draw on the CRF. If the date or time of the last dose is unclear, provide the participant's best approximation.
    h. Serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH).
    i. Fractionate bilirubin, if total bilirubin >2xULN
    j. Assess for peripheral eosinophilia
    k. Assess for hypoxic/ischemic hepatopathy; and biliary tract disease

The following are required for patients who meet the stopping criteria for both ALT and bilirubin OR experiences clinical symptoms of hepatitis:

1. Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies and quantitative total immunoglobulin G (IgG or gamma globulins).
2. If required evaluation of competing undiagnosed liver disease (hemochromatosis, Wilson's disease, alpha-1 anti-trypsin deficiency)
3. Serum acetaminophen levels OR serum acetaminophen adducts by HPLC assay (quantifies potential acetaminophen contribution to liver injury in participants with definite or likely acetaminophen use in the preceding week).
4. Liver imaging (ultrasound, magnetic resonance, or computerized tomography) to evaluate liver disease.
5. A Liver Biopsy

Treatment with investigational agent could be re-initiated if these abnormalities stabilize, return to pre-trial baseline, or normalize.

Investigational agent must be permanently discontinued and patient must be followed until the clinical and laboratory abnormalities stabilize or normalize if the following criteria are met:

1) If close monitoring of a patient is not possible. or
2) Any elevation of direct bilirubin > ULN, regardless of ALT or AST levels, AND with indicators of immunological reaction (i.e., rash or >5% eosinophilia), or appearance of nausea, vomiting, right upper quadrant pain (symptoms consistent with clinical hepatitis)
3) Any development of recurrent hepatotoxicity (as outlined in this table) upon rechallenge.
4) Any hepatic decompensation as
    - New onset ascites or worsening of ascites (requiring increase in drug therapy or requiring surgical intervention such as paracentesis).
    - Variceal bleeding documented by endoscopy
    - Spontaneous bacterial peritonitis documented by positive culture
    - Hepatic encephalopathy
    - Hepatorenal syndrome (Type 1 or 2)
    - Porto-pulmonary hypertension
    - Hepato-pulmonary hypertension
    - Any liver related event that leads to hospitalization or G4 event.

APPENDIX E

Alcohol and Tobacco Use Assessment
ALCOHOL ASSESSMENT – BASELINE

Instructions:

For the following questions about drinking alcoholic beverages, a drink means a 12 oz. beer, a 5 oz. glass of wine, or one and a half ounces of liquor.

When a number is requested in the response, please enter a whole number (i.e. "4") and not a range or fraction of a number.

1. In your entire life, have you had at least 12 drinks of any kind of alcoholic beverage?
   - ☐ Yes
   - ☐ No **(End)**
   - ☐ Refused **(End)**
   - ☐ Don't know/Not sure

2. In the past 12 months, on average, how often did you drink any type of alcoholic beverage?
   _____ (Enter the number of days you drank based on the timeframe checked below. Enter 0 if you never drank and skip to Question 6.)
   - ☐ Week
   - ☐ Month
   - ☐ Year
   - ☐ Refused
   - ☐ Don't know/Not sure

3. In the past 12 months, on those days that you drank alcoholic beverages, on average, how many drinks did you have per day?
   _____ (Enter the average number of drinks per day)

   - ☐ Refused
   - ☐ Don't know/Not sure

4. In the past 12 months, on how many days did you have 5 or more drinks of any alcoholic beverage?
   _____ (Enter the number of days you had 5 or more drinks, or enter 0 if none.)

   - ☐ Refused
   - ☐ Don't know/Not sure

5. Was there ever a time or times in your life when you drank 5 or more drinks of any kind of alcoholic beverage almost every day?
   - ☐ Yes
   - ☐ No
   - ☐ Refused
   - ☐ Don't know/Not sure

6. If you do not currently drink alcoholic beverages, but did in the past, how long has it been since you last drank regularly?
   - ☐ Within the past month (0 to 1 month ago)
   - ☐ Between 1 and 3 months (1 to 3 months ago)
   - ☐ Between 3 and 6 months (3 to 6 months ago)
   - ☐ Between 6 and 12 months (6 to 12 months ago)
   - ☐ Between 1 and 5 years (1 to 5 years ago)
   - ☐ Between 5 and 15 years (5 to 15 years ago)
   - ☐ More than 15 years ago
   - ☐ Don't know/Not sure
   - ☐ Never drank regularly

7. At the heaviest point, either now or in the past, on the days when you drank, about how many drinks did you drink a day on the average?
   _____ (Enter the number of drinks a day)

   - ☐ Refused
   - ☐ Don't know/Not sure

8. How many years have you been drinking (or did drink) regularly?
   _____ years

   - ☐ Refused
   - ☐ Don't know/Not sure

9. At what age did you begin drinking regularly?
   _____ years of age

   - ☐ Refused
   - ☐ Don't know/Not sure

10. What type(s) of alcohol do you drink? (Mark ALL that apply)
    - ☐ Wine
    - ☐ Liquor
    - ☐ Beer
    - ☐ Wine cooler

Coordinator Signature _____ Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

APPENDIX F

Alcohol and Tobacco Use Assessment
TOBACCO ASSESSMENT – BASELINE

Instructions:

When a number is requested in the response, please enter a whole number (i.e. "4") and not a range or fraction of a number.

Section A. Basic Cigarette Use Information

1. Have you smoked at least 100 cigarettes (5 packs = 100 cigarettes) in your entire life?
   - ☐ Yes
   - ☐ No → **Skip to Section B**
   - ☐ Don't know/Not sure → **Skip to Section B**

2. How old were you when you first smoked a cigarette (even one or two puffs)?
   _____ Years old

3. How old were you when you first began smoking cigarettes regularly?
   _____ Years old
   - ☐ Check here if you have never smoked cigarettes regularly.

4. How many total years have you smoked (or did you smoke) cigarettes? Do not count any time you may have stayed off cigarettes.
   _____ Years (If you smoked less than one year, write "1.")

5. On average when you have smoked, about how many cigarettes do you (or did you) smoke a day? (A pack usually has 20 cigarettes in it).
   _____ Number of cigarettes per day

6. Do you NOW smoke cigarettes?
   - ☐ Everyday
   - ☐ Some days
   - ☐ Not at all → **Skip to question 8**

7. How soon after you wake up do you smoke your first cigarette?
   - ☐ Within 30 minutes
   - ☐ After 30 minutes

8. How long has it been since you last smoked a cigarette (even one or two puffs)?
   First check which one of the following choices applies to you. Then, if applicable, write a number on the line for how many days, weeks, months, or years it has been since your last cigarette.
   - ☐ I smoked a cigarette today (at least one puff)
   - ☐ 1-7 days → Number of days since last cigarette _____
   - ☐ Less than 1 month → Number of weeks since last cigarette _____
   - ☐ Less than 1 year → Number of months since last cigarette _____
   - ☐ More than 1 year → Number of years since last cigarette _____
   - ☐ Don't know/Don't remember

Section B. Use of Other Forms of Tobacco

9. Have you ever used other forms of tobacco, not including cigarettes?
   - ☐ Yes
   - ☐ No → **Skip to Section C**

10. How often do you/did you use other forms of tobacco?
   - ☐ Every day → Number of times per day _____
   - ☐ Some days → Number of days _____ per ☐ Week ☐ Month ☐ Year

11. Which of the following products have you ever used regularly?
   Check all that apply
   - ☐ Cigarettes
   - ☐ E-cigarettes or other electronic nicotine delivery system
   - ☐ Traditional cigars, cigarillos or filtered cigars
   - ☐ Pipes
   - ☐ Hookah
   - ☐ Clove cigarettes or kreteks
   - ☐ Bidis
   - ☐ Smokeless tobacco, like dip, chew, or snuff
   - ☐ Snus
   - ☐ Paan with tobacco, gutka, zarda, khaini
   - ☐ Other, Please specify:_____

12. If you do not currently use other forms of tobacco, but did in the past, how long has it been since you last used other forms of tobacco regularly?
   - ☐ Within the past month (0 to 1 month ago)
   - ☐ Between 1 and 3 months (1 to 3 months ago)
   - ☐ Between 3 and 6 months (3 to 6 months ago)
   - ☐ Between 6 and 12 months (6 to 12 months ago)
   - ☐ Between 1 and 5 years (1 to 5 years ago)
   - ☐ Between 5 and 15 years (5 to 15 years ago)
   - ☐ More than 15 years ago
   - ☐ Don't know/Not sure
   - ☐ Never used other forms of tobacco regularly

Section C. Second-Hand Smoke Exposure

13. Are you currently living with a smoker?
   - ☐ Yes
   - ☐ No

14. In the past 30 days, have you lived in a place where other people smoked cigarettes indoors?
   - ☐ Yes
   - ☐ No

15. In the past 30 days, have you worked in a place where other people smoked cigarettes indoors?
    - ☐ Yes
    - ☐ No

16. Thinking of all your childhood and adult years, have you ever lived in a place where other people smoked cigarettes indoors?
    - ☐ Yes In total, for about how many years?_____ If less than 1, write "1."
    - ☐ No

17. Thinking of all the years you have worked, have you ever worked in a place where other people smoked cigarettes indoors?
    - ☐ Yes → In total, for about how many years?_____ If less than 1, write "1."
    - ☐ No

Coordinator Signature _____ Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

APPENDIX G

Alcohol and Tobacco Use Assessment
ALCOHOL ASSESSMENT - FOLLOW-UP

Instructions:
For the following questions about drinking alcoholic beverages, a drink means a 12 oz. beer, a 5 oz. glass of wine, or one and a half ounces of liquor.

When a number is requested in the response, please enter a whole number (i.e. "4") and not a range or fraction of a number.

1. During the past 30 days, did you drink any alcoholic beverages?
   - ☐ Yes
   - ☐ No **(End)**
   - ☐ Refused **(End)**
   - ☐ Don't know/Not sure

2. During the past 30 days, how many days per week or per month did you drink any alcoholic beverages, on the average?
   _____ (Enter number of days you drank based on the timeframe checked below. Enter 0 if you did not drink.)
   - ☐ Week
   - ☐ Month
   - ☐ Refused
   - ☐ Don't know/Not sure

3. On the days when you drank, on average, about how many drinks did you have?
   _____ (Enter the average number of drinks you had per day.)
   - ☐ Refused
   - ☐ Don't know/Not sure

4. In the past 30 days, on how many days did you have 5 or more drinks per day?
   _____Number of times
   - ☐ None
   - ☐ Do not know/Not sure

Coordinator Signature _____ Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

APPENDIX H

Alcohol and Tobacco Use Assessment
TOBACCO ASSESSMENT - FOLLOW-UP

Instructions:

When a number is requested in the response, please enter a whole number (i.e. "4") and not a range or fraction of a number.

1. Do you NOW smoke cigarettes?
   - ☐ Everyday
   - ☐ Some days
   - ☐ Not at all → **Skip to Question 3.**
   - ☐ Never smoked → **Skip to Question 4.**

2. On average, when you smoked, about how many cigarettes do you (or did you) smoke a day? (A pack usually has 20 cigarettes in it).

   _____ Number of cigarettes per day

3. How long has it been since you last smoked a cigarette (even one or two puffs)?
   First check which one of the following choices applies to you. Then, if applicable, write a number on the line for how many days, weeks, months, or years it has been since your last cigarette.
   - ☐ I smoked a cigarette today (at least one puff)
   - ☐ 1-7 days → Number of days since last cigarette _____
   - ☐ Less than 1 month → Number of weeks since last cigarette _____
   - ☐ Less than 1 year → Number of months since last cigarette _____
   - ☐ More than 1 year → Number of years since last cigarette _____
   - ☐ Don't know/Don't remember

4. Since your last visit, have you used other forms of tobacco, not including cigarettes?
   - ☐ Yes
   - ☐ No (**End)**

5. How often do you/did you use other forms of tobacco?
   - ☐ Every day → Number of times per day _____
   - ☐ Some days → Number of days _____ per ☐ Week ☐ Month ☐ Year

6. Since your last visit, which of the following products have you used? ***Check all that apply***
   - ☐ Cigarettes
   - ☐ E-cigarettes or other electronic nicotine delivery system
   - ☐ Traditional cigars, cigarillos or filtered cigars
   - ☐ Pipes
   - ☐ Waterpipe
   - ☐ Hookah
   - ☐ Clove cigarettes or kreteks
   - ☐ Bidis
   - ☐ Smokeless tobacco, like dip, chew, or snuff
   - ☐ Snus
   - ☐ Paan with tobacco, gutka, zarda, khaini
   - ☐ Other, Specify_____

7. If you do not currently use other forms of tobacco, but did in the past, how long has it been since you last used other forms of tobacco regularly?
   - ☐ Within the past month (0 to 1 month ago)
   - ☐ Between 1 and 3 months (1 to 3 months ago)
   - ☐ Between 3 and 6 months (3 to 6 months ago)
   - ☐ Between 6 and 12 months (6 to 12 months ago)
   - ☐ Between 1 and 5 years (1 to 5 years ago)
   - ☐ Between 5 and 15 years (5 to 15 years ago)
   - ☐ More than 15 years ago
   - ☐ Don't know/Not sure
   - ☐ Never used other forms of tobacco regularly

The following instructions pertain to questions 8 - 10. During each of the following time frames, please indicate whether you smoked cigarettes every day, some days, or not at all.

8. During study treatment
   - ☐ Smoked every day
   - ☐ Smoked some days
   - ☐ Did not smoke at all
   - ☐ Don't know/not sure
   - ☐ Not applicable

9. After the end of study treatment
   - ☐ Smoked every day
   - ☐ Smoked some days
   - ☐ Did not smoke at all
   - ☐ Don't know/not sure
   - ☐ Not applicable (I have not completed the study treatment)

10. Since your last visit to this clinic
    - ☐ Smoked every day
    - ☐ Smoked some days
    - ☐ Did not smoke at all
    - ☐ Don't know/not sure

Coordinator Signature _____ Date __ __ / __ __ / __ __ __ *(MM/DD/YYYY)*

APPENDIX I

Resources

National and local resources to help with alcohol abuse and alcoholism

NIAAA's online guide ***Treatment for Alcohol Problems: Finding and Getting Help*** is written for individuals, and their family and friends, who are looking for options to address alcohol problems. It is intended as a resource to understand what treatment choices are available and what to consider when selecting among them.
https://pubs.niaaa.nih.gov/publications/treatment/treatment.htm

Other resources:

National Institute on Alcohol Abuse and Alcoholism www.niaaa.nih.gov
301–443–3860

National Institute on Drug Abuse www.nida.nih.gov
301–443–1124

National Clearinghouse for Alcohol and Drug Information www.samhsa.gov
1–800–729–6686

Substance Abuse Treatment Facility Locator www.findtreatment.samhsa.gov
1–800–662–HELP

Alcoholics Anonymous (AA) www.aa.org
212–870–3400 or check your local phone directory under "Alcoholism"

Moderation Management www.moderation.org
212–871–0974

Secular Organizations for Sobriety www.sossobriety.org
323–666–4295

SMART Recovery www.smartrecovery.org
440–951–5357

Women for Sobriety www.womenforsobriety.org
215–536–8026

Al-Anon Family Groups www.al-anon.alateen.org
1–888–425–2666 for meetings

Adult Children of Alcoholics www.adultchildren.org
310–534–1815

National and local resources to help with quitting smoking

NCI's Smokefree.gov offers science-driven tools, information, and support that has helped smokers quit. You will find state and national resources, free materials, and quitting advice from NCI.

Smokefree.gov was established by the Tobacco Control Research Branch (http://www.cancercontrol.cancer.gov/tcrb/) of NCI, a component of the National Institutes of Health, in collaboration with the Centers for Disease Control and Prevention and other organizations.

Publications available from the Smokefree.gov Web site include the following:

- Clearing the Air: Quit Smoking Today (http://smokefree.gov/sites/default/files/pdf/clearing-the-air-accessible.pdf) for smokers interested in quitting.
- Clear Horizons (http://www.smokefree.gov/landing.aspx?rid=2) for smokers over age 50.
- Forever Free™ (http://www.smokefree.gov/landing.aspx?rid=3) for smokers who have recently quit.
- Forever Free for Baby and Me™, in English (http://www.smokefree.gov/landing.aspx?rid=4) and Spanish (http://www.smokefree.gov/landing.aspx?rid=5), for pregnant smokers who have recently quit.
- Pathways to Freedom: Winning the Fight Against Tobacco (http://www.cdc.gov/tobacco/quit_smoking/how_to_quit/pathways/index.htm**)** for African American smokers.

NCI's Smoking Quitline at **1–877–44U–QUIT (1–877–448–7848)** offers a wide range of services, including individualized counseling, printed information, referrals to other resources, and recorded messages. Smoking cessation counselors are available to answer smoking-related questions in English or Spanish, Monday through Friday, 8:00 a.m. to 8:00 p.m., Eastern time. Smoking cessation counselors are also available through LiveHelp (https://livehelp.cancer.gov/), an online instant messaging service. LiveHelp is available Monday through Friday, 8:00 a.m. to 11:00 p.m., Eastern time.

Your state has a toll-free telephone quitline. Call **1–800–QUIT–NOW (1–800–784–8669)** to get one-on-one help with quitting, support and coping strategies, and referrals to resources and local cessation programs. The toll-free number routes callers to state-run quitlines, which provide free cessation assistance and resource information to all tobacco users in the United States. This initiative was created by the Department of Health and Human Services (http://www.hhs.gov/). For more information about quitlines, speak to an expert (http://smokefree.gov/talk-to-an-expert) on the Smokefree.gov Web site.