HRP-592 - Protocol for Human Subject Research with Use of Test Article(s)

Protocol Title:

Study of Angelica gigas dietary supplement (Cogni.Q) potential effects on human immune cells

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Clinicaltrials.gov Registration #: Pending

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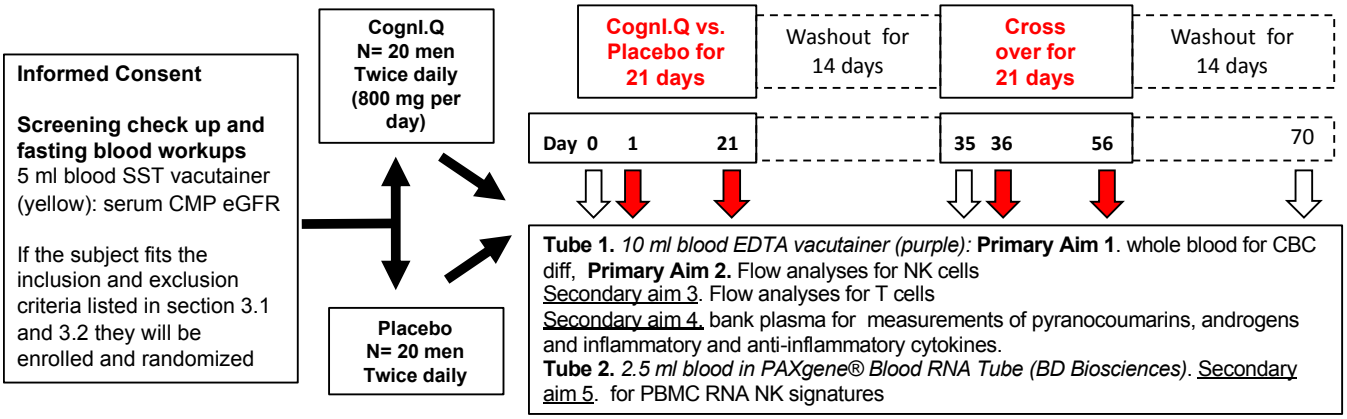
1.0 Objectives

1.1 Study Objectives

The purpose of this pilot clinical trial is to test that daily intake of Korean *Angelica gigas* Nakai (AGN) dietary supplement (Cogni.Q, Quality of Life Laboratories, Purchase, NY) may boost human innate immune functions. Cogni. Q is a dietary supplement which is commercially available for anyone to purchase without a prescription for memory improvement.

The hypothesis to be tested is that daily consumption of Cogni. Q supplement leads to increased number and/or activation of neutrophils and Natural Killer (NK) cells in healthy men.

To test the hypothesis, we propose a double-blinded, placebo-controlled trial design (See scheme).



1.2 Primary Study Endpoints

The primary endpoints/Aims are:

Primary Aim 1 (Tube 1). whole blood for CBC diff.

Primary Aim 2 (Tube 1). Flow analyses for NK cell counts.

1.3 Secondary Study Endpoints

Secondary objectives are:

Aim 3 (Tube 1): Flow analyses for T cell counts.

Aim 4 (Tube 1): To determine plasma concentration of pyranocoumarins (as compliance to Cogni.Q/placebo exposure); plasma protein profiling for androgens, and inflammatory/anti-inflammatory cytokines.

Aim 5 (tube 2): To profile the NK mRNA signature by RNA-seq transcriptomics using RNA prepared from Pax Gene tube-preserved whole blood collected on each visit.

2.0 Background

2.1 Scientific Background and Gaps

Korean *Angelica gigas* Nakai (AGN)-containing products (e.g., Cogni.Q to be studied in the current protocol, AcheAction, Decursinol-50, Fast-Acting Joint Formula, EstroG-100/Profemin) are marketed as dietary supplements for memory improvement, pain relief and women's health especially for menopausal symptom management. Except for the 3-herbal EstroG-100/Profemin (AGN, Cynanchum wilfordii, Phlomis umbrosa) [1], these dietary supplements have not been tested in Americans in rigorously controlled human trials. *Their benefit to US consumers is an open question.* Because of the differences between Koreans and US residents in genetic background, life styles, food patterns and preferences, *etc.,* human studies in the US are needed to ensure health benefits are translatable to American consumers.

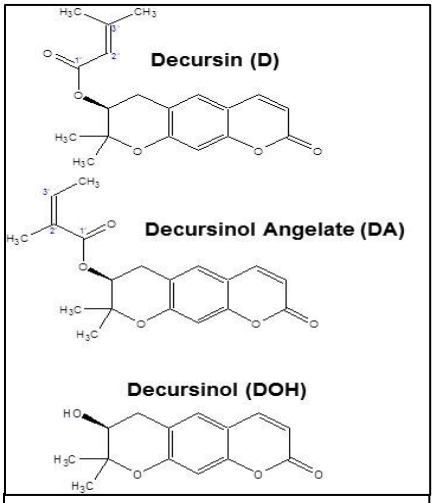


Fig. 1 Chemical structures of pyranocoumarins from AGN

Decursin (D) and its isomer decursinol angelate (DA) (**Fig. 1**) are the major marker compounds in the ethanol extract of AGN root [2]. In rodent models, we and others have shown that D/DA are rapidly converted to decursinol (DOH) after gavage or i.p. injection [2-4]. Our "omic" analyses of the TRAMP (the transgenic adenocarcinoma of the mouse prostate model) neuroendocrine carcinomas suggest **immune enhancement** by AGN [5]. However, a crucial gap in translating our rodent mechanistic studies to human benefits is whether humans metabolize AGN and D/DA to DOH as in these rodent models.

To fill in that gap, we completed a 20-subject single dose-PK study in Adult Men and Women with normal liver and kidney function (per CMP eGFR) in Amarillo, TX residents (age 21-58 years, 65% Caucasian, 20% Hispanic, 15% Asian Indian) with AGN dietary supplement Cogni.Q (purchased from Quality of Life Laboratories, Purchase, NY) [6]. For dosage, each person swallowed 4 vegicaps (800 mg AGN) at time 0, the recommended daily dose by manufacturer. Cogni.Q is marketed for promoting cognitive agility and healthy brain function. **Fig. 2** shows plasma D/DA and DOH vs. time curves. The human $AUC_{0\text{-}48h}$ for DOH (27579 h.nmol/L) is much higher than for DA (335 h.nmol/L) and D (37 h.nmol/L), supporting extensive conversion from D/DA→DOH. The human PK parameters recapitulate patterns in rodent models ([2, 3], substantiating their biological relevance for mechanistic insights.

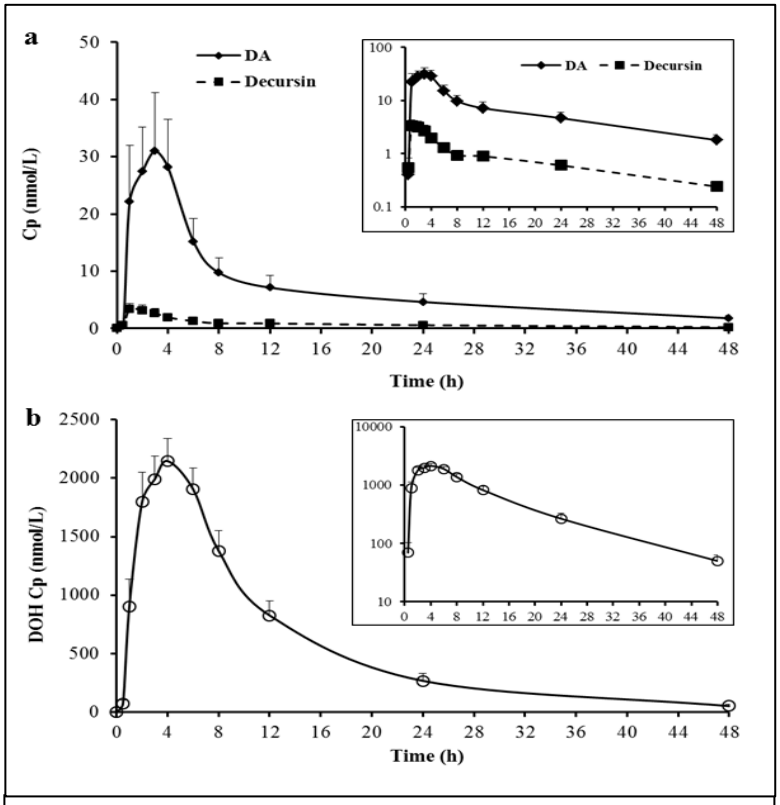


Fig. 2 Human plasma (**a**) D,DA& (**b**) DOH concentration (nM) vs. time. Insets are data presented on log scale. Mean + sem

Significantly, <u>as secondary endpoint parameters,</u> the neutrophil counts in these human subjects were increased by 71% at 24h post-dose vs. pre-dose and the natural killer cell (NK) mRNA signature (+60~90%) in their peripheral blood mononuclear cells (PBMC) (**See 2.2 previous data**). Neutrophils and NK cells belong to two lineages of immune cells that make up our innate immune defense. Neutrophils (myeloid lineage) fight against bacterial infection whereas NK cells (lymphoid lineage) not only kill virus-infected cells but also recognize and kill cancer cells, serving as a crucial immune surveillance against malignancy in our body.

We **hypothesize** that daily intake of AGN dietary supplement (Cogni.Q) increases the number and/or activities of neutrophils and NK cells, and in turn may boost human innate immune function against bacterial and viral infections as well as cancer risk. As an initial effort to test our hypothesis, the goal of this pilot clinical trial is to delineate the Cogni.Q supplement specific innate immune enhancement activity in healthy men in the Hershey-Harrisburg area using a **double-blinded, placebo-controlled trial design** conducted through Penn State Clinical Translational Science Institute's Clinical Research Center (CTSI CRC) and College of Medicine laboratory facilities (See scheme **Fig. 1**).

2.2 Previous Data

Neutrophils and Natural killer (NK) cells are first line immune cells that defend against viral and bacterial infections as well as cancer.

These two cell lineages of the innate immune system mount acute defense against invading microorganisms and regulate inflammation. **Neutrophils** phagocytose and kill engulfed bacteria by enzymatic (e.g., lysozymes to degrade bacterial wall, proteases to degrade cellular proteins) and chemical means (hypochlorite, ROS) [7, 8]. Neutrophils are abundant at sites of infection and inflammation and are critical for resolving inflammation [8]. **NK cells** search out and kill virus-infected cells and cancer cells that have stress-induced dysregulation of cell surface markers [9]. Cell killing by NK cells is mediated by granzyme serine-proteases released from cytoplasmic granules that enter target cells [10] through a multimeric complex made up of Granzyme B, Perforin, and Granulysin to trigger apoptosis through caspases. Therefore, enhancing the number and/or activities of these two innate immune cells through herbal supplements would be non-invasive and practical for fending off bacterial and viral infections and may shorten the time to resolve infection-driven illnesses. As chronic inflammation is causally linked to cancer the above benefits as well as NK cancer killing action could translate into cancer risk reduction and control.

Table 1. CBC panel (24h/0h ratio)

CBC parameter	Ratio 24h/0h
ALBUMIN	NO CHANGE
GLOBULIN	NO CHANGE
white blood cells	1.21 X
red blood cells	NO CHANGE
hemoglobin	NO CHANGE
hematocrit	NO CHANGE
MCV	NO CHANGE
MCH	NO CHANGE
MCHC	NO CHANGE
RDW	NO CHANGE
Platelet	1.13 X
Absolute neutrophils	1.71 X
Absolute lymphocytes	NO CHANGE
Absolute monocytes	NO PATTERN
Absolute eosinophils	NO PATTERN
Absolute basophils	NO PATTERN

All fold changes p<0.05

Table 2 Neutrophil count (per microliter) in men vs. women

	Neutrophil count per/μL				Men vs. Women	Correlation with age	Correlation with weight
	All Subjects (n=19)*		Men (n=10)	Women (n=9)			
	Mean	SD	Mean	Mean	t-test, p	R^{2**}	R^{2**}
Baseline	2065	1183	2027	2167	0.738	0.0438	<0.0001
24 h post Cogni.Q	2930	1396	3012	2952	0.952	0.0226	0.0151
paired t-test, p	0.0004		0.0108	0.0262			

*Missing value from 1 female. **R^2-coefficient of determination for linear regression

Measurable changes of human neutrophil counts after a single Cogni.Q supplement As part of our published PK study [6], we collected complete blood cell (CBC diff, Quest Diagnostics) panel data on fasting blood at baseline and 24 h. To our surprise, total white blood cell (WBC) counts were 21% higher at 24 h (Table 1) and neutrophils were much higher (**Table 1**, individual ratio +71%; **Table 2**, group mean counts +42%).

Importantly, 7/20 subjects had absolute neutrophil counts below 1500 (low normal reference value) at baseline (S1, 232; S4, 598; S5, 673; S13, 821; S15, 1280; S18, 833; S20, 1485). At 24 h post-dose, 2 subjects remained below this cut off value (S1, 232 → **800**; S13, 821→ **1468**), the other 5 subjects all improved above 1500 (S4, **1847**; S5, **1665**; S15, **1783**; S18, **1721**; S20, **1998**). These data suggest that a **substantial proportion (~35%) of "healthy" subjects may have had neutropenia of varying degree**, including severe neutropenia (<500). Thus, the potential use of Cogni.Q dietary

supplementation to correct neutropenia affordably and non-invasively is implicated vs. the case of injection of granulocyte colony-stimulating factors (G-CSFs).

Neither baseline nor 24 h counts correlated with age (range 21-58 yr) and body weight (range 115-200 lb) of the subjects (**Table 2**, R^2-coefficient of determination indicates no contribution to neutrophil variance). Furthermore, gender did not affect the baseline or 24 h count (**Table 2**).

NK-transcriptome changes in human peripheral blood mononuclear cells (PBMC) after Cogni.Q We isolated RNA from PBMC using QIAamp® RNA Blood Mini kit (QIAGEN) and profiled mRNA expression from 6 subjects using Illumina Human HT-12 BeadChip array. All RNA labeling and hybridization were performed as before [5]. **Table 3** highlights that **NK-related and immune signature genes were prominently** affected**.** We used real-time RT-PCR as before [5] to verify select genes, normalized to housekeeping gene *ß-actin* (**Fig. 2)**. NK mRNA expression markers were **+60~90%** higher for 24h post-dose vs. baseline (n = 6, p<0.01) (**Fig. 2A**). However, not all genes changed in the same direction (i.e., unlikely systematically biased). The inflammatory cytokine gene *IL-8* in PBMC was reduced (Fig. 2A). By 48 h, *granulysin (GNLY)* returned to baseline whereas *KLRF1* was still elevated (**Fig. 2B**), suggesting temporal response differences among NK molecules. We will use placebo and a week of daily supplement to rigorously test and validate the Cogni.Q-specific enhancement of the NK-signature at both cellular and molecular levels.

Table 3. Enriched pathways 24h post-Cogni.Q vs. baseline in PBMC mRNA by microarray profiling

Pathway name	Pathway source	# genes	Overlapping genes	P values
Natural killer cell mediated cytotoxicity	KEGG	5	KLRD1;GZMB;KIR2DL3;KIR2DL4;PRF1	1.06E-05
Graft-versus-host disease	KEGG	4	KLRD1;GZMB;KIR2DL3;PRF1	1.95E-06
IL12-mediated signaling events (NK)	PID	4	EOMES;CCL4L2;GZMB;GZMA	1.18E-05

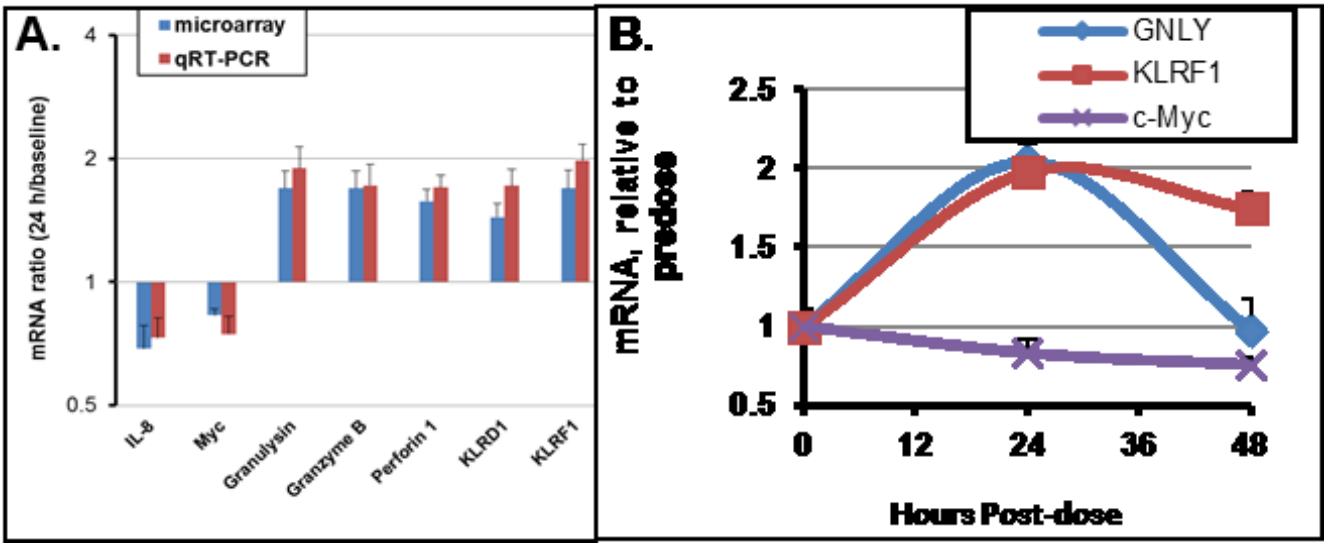


Fig. 2. (A) PBMC mRNA changes 24h post-dose/baseline (n=6). **(B)** Time course of selected genes

2.3 Study Rationale

Based on the literature with AGN animal studies and our single dose PK study which showed, as secondary readouts, an enhancement of immune cells (neutrophil cells) and NK mRNA signatures, the current study was designed to rigorously establish the impact of Cogni.Q on 2 types of human innate immune cells by placebo-controlled and crossover design. The research is novel because the immune promotion activity has not been reported in any human population before. Positive data from our proposed trial will be the first of its kind to support acute enhancement in healthy US residents of these innate immune cells by an AGN dietary supplement. Both public health and clinical therapeutic applications could be developed. Clinical applications for treating human neutropenia of various causes including many cancer therapeutics should be of great further research interest.

3.0 Inclusion and Exclusion Criteria

Note: Our pilot clinical trial will be only focused on men due to three main reasons: i) our ongoing focus on male-only prostate cancer prevention and therapy by AGN, ii) the preliminary data with a single dose of AGN supplement had suggested that both men and women responded to the same extent for boosting up neutrophils (**Table 2**); and iii) menstrual cycle variations and potential pregnancy in women over a two-month study may complicate acquisition of data to address our hypothesis in our pilot trial of a small study population. The information gained from this pilot study will be used for the future trials with more study subjects and will include both genders.

3.1 Inclusion Criteria

- Male subjects 21 to 65 years of age
- Subjects weighing between 100 to 240 pounds; their body mass index (BMI) should be in the range of 19 to 30
- Subjects having normal hepatic, renal function as assessed by history, physical and clinical chemistry analysis (CMP eGFR, see supporting document for normal reference ranges).
- Subjects with normal blood pressure (systolic below 120 mm Hg and diastolic below 80 mm Hg)

3.2 Exclusion Criteria

- Subjects positive for HIV, HBV and HCV (self-reported)
- Subjects taking any kind of prescription medications regularly or within 10 days of the study will be excluded. Therefore, subjects with diabetes, major cardiovascular diseases, cancer, severe hypertension, severe hepatic cirrhosis or cirrhosis of the liver, and kidney disease (self-reported) will be excluded.
- Subjects using tobacco products, nicotine patches and excessive alcohol
- Subjects taking dietary or herbal supplements that contain AGN (e.g. Cogni.Q, Decursinol-50, Ache Action, Fast-Acting Joint Formula, EstroG-100/Profemin) within 10 days of the study.
- Non-English-speaking subjects

3.3 Early Withdrawal of Subjects

3.3.1 Criteria for removal from study

Although we expect Cogni.Q supplement-caused adverse event (AE) or serious adverse event (SAE) to be extremely unlikely, nevertheless, the clinical research staff and study physician will be responsible for detecting and documenting events that meet protocol defined criteria as an adverse event (AE) or a serious adverse event (SAE) and assist the PI in reporting.

An AE is defined as an untoward medical occurrence in a subject temporally associated with the use of a medicinal study product whether or not it is considered related to the medicinal product

An AE can be any unfavorable and unintended symptom or sign (abnormal laboratory finding) or disease temporally related to the use of a medicinal product. This can include the following:

- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or grade of the condition
- New conditions detected or diagnosed after study treatment administration even though it may have been present prior to the start of the study
- Signs, symptoms or the clinical sequelae of a suspected interaction
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication.

An SAE is defined as the following:

- Results in death
- Is life-threatening
- Requires hospitalization
- Results in disability/incapacity

Any subject who experiences an adverse event may be withdrawn from the supplement (either arm) and/or the study at any time and for any reason.

3.3.2 Follow-up for withdrawn subjects

A subject can withdraw from trial at any time of the study. Withdrawn subjects will be followed up by study staff with phone calls or electronic media within 2 weeks.

4.0 Recruitment Methods

4.1 Identification of subjects

CTSI CRC healthy volunteer database will be used to solicit study subjects plus multiple advertising venues of trial participation opportunity will be made in the Hershey and Harrisburg metro area, including CTSI website and CTSI community engagement outreach networks, Penn State STUDYfinder, local civic institutions and grocery supermarkets.

4.2 Recruitment process

After a “scripted” phone screening containing the verbal consent (see supporting documents for phone script), eligible male subjects will be invited to an initial visit to CRC for informed consent. Once the written informed consent has been obtained, the subject’s eligibility will be verified as per the procedure described in section 7.2. If the subject fits all the inclusion and exclusion requirements listed in section 3.1 and 3.2, he will be contacted by the research staff to see whether he wants to participate in the trial.

4.3 Recruitment materials

For recruitment material the Office of Marketing and Communications has prepared a draft flyer for the committee review (see supporting documents). Moreover, flyer will be also displayed on the television throughout the campus and social media advertisement. Furthermore, STUDYfinder will be used for recruitment purposes.

4.4 Eligibility/screening of subjects

Verbal informed consent will be obtained before asking potential subjects with eligibility questions on phone.

5.0 Consent Process and Documentation

5.1 Consent Process

5.1.1 Obtaining Informed Consent

5.1.1.1 Timing and Location of Consent

After verbal consent during phone screening, a written Informed consent will be obtained when the subjects come for their initial (screening) visit at CRC.

5.1.1.2 Coercion or Undue Influence during Consent

In order to minimize the possibility of coercion or undue influence in the consent process, a member of the study team will explain the details about the trial during their initial screening visit. Potential subjects will then be given time to read and sign the consent in a private setting at CRC.

5.1.2 Waiver or alteration of the informed consent requirement

Not applicable

5.2 Consent Documentation

5.2.1 Written Documentation of Consent

See attached supporting documents

5.2.2 Waiver of Documentation of Consent (Implied consent, Verbal consent, etc.)

We request a waiver of documentation of consent for phone screen procedure.

5.3 Consent – Other Considerations

5.3.1 Non-English Speaking Subjects

NOT APPLICABLE

5.3.2 Cognitively Impaired Adults

NOT APPLICABLE

5.3.2.1 Capability of Providing Consent

NOT APPLICABLE

5.3.2.2 Adults Unable To Consent

NOT APPLICABLE

5.3.2.3 Assent of Adults Unable to Consent

NOT APPLICABLE

5.3.3 Subjects who are not yet adults (infants, children, teenagers)

5.3.3.1 Parental Permission

NOT APPLICABLE

5.3.3.2 Assent of subjects who are not yet adults

NOT APPLICABLE

6.0 HIPAA Research Authorization and/or Waiver or Alteration of Authorization

6.1 Authorization and/or Waiver or Alteration of Authorization for the Uses and Disclosures of PHI

Check all that apply:

☐ **Not applicable, no identifiable protected health information (PHI) is accessed, used or disclosed in this study.** *[Mark all parts of sections 6.2 and 6.3 as not applicable]*

X **Authorization will be obtained and documented as part of the consent process.** *[If this is the only box checked, mark sections 6.2 and 6.3 as not applicable]*

☐ **Partial waiver is requested for recruitment purposes only (Check this box if patients' medical records will be accessed to determine eligibility before consent/authorization has been obtained).** *[Complete all parts of sections 6.2 and 6.3]*

☐ **Full waiver is requested for entire research study (e.g., medical record review studies).** *[Complete all parts of sections 6.2 and 6.3]*

☐ **Alteration is requested to waive requirement for written documentation of authorization (verbal authorization will be obtained).** *[Complete all parts of sections 6.2 and 6.3]*

6.2 Waiver or Alteration of Authorization for the Uses and Disclosures of PHI
NOT APPLICABLE

6.2.1 Access, use or disclosure of PHI representing no more than a minimal risk to the privacy of the individual

6.2.1.1 Plan to protect PHI from improper use or disclosure
Not applicable

6.2.1.2 Plan to destroy identifiers or a justification for retaining identifiers
Not applicable

6.2.2 Explanation for why the research could not practicably be conducted without access to and use of PHI
Not applicable

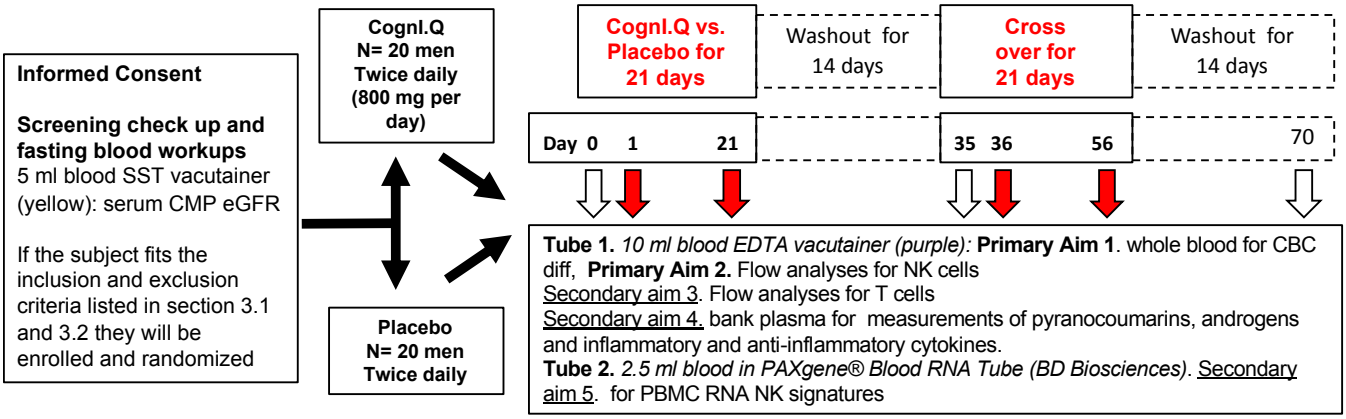
6.2.3 Explanation for why the research could not practicably be conducted without the waiver or alteration of authorization
Not applicable

6.3 Waiver or alteration of authorization statements of agreement
Not applicable

7.0 Study Design and Procedures

7.1 Study Design

We plan on conducting a double-blinded, placebo-controlled, crossover trial design for testing our hypothesis.



7.2 Study Procedures

After phone screening and if they meet the criteria of the study inclusion and exclusion, they will be asked to come to CRC facility for a screening visit. Screening visit procedure is described below:

Screening Visit:

- Subjects will be asked to read and sign a consent document before any study-specific tests or procedures are performed. Subjects will be given a signed and dated copy of the consent form for their records.
- They will complete a health history questionnaire (See Supporting Material) regarding their medical history past and present, any prescription and/or non-prescription medications they are taking including herbal preparations, multivitamins, supplements. They will also be asked about any underlying medical issues, prior surgeries which might affect the absorption of Cogni.Q such as gastric bypass/sleeve/banding surgery, alcohol and smoking histories.
- They will be required to undergo a physical examination which will include recording their height, weight, blood pressure, heart rate, as well as an examination of their head/neck area, lungs, heart, abdomen, extremities, neurological status.
- Blood will be collected from a vein in their arm (approximately 1 teaspoon or 5 mL) for common blood tests to test their health status.
- This screening visit will take approximately 1 hour.

Based on data collected during the screening visit and lab report, study physicians will certify participation eligibility as per the inclusion and exclusion criteria described in section 3.1 and 3.2. A study coordinator will contact the eligible subjects to schedule their study visits. A subject number will be assigned to each subject after they have scheduled their study visits.

The study team will fax a prescription [that has been signed by a study physician on the protocol] to the Investigational Drug Service Pharmacy (IDS). The IDS Pharmacy will refer to the randomization table (provided in advance by the study biostatistician) and dispense the investigational product **(IP)**(Cogni.Q 200mg/ Placebo Capsules) that corresponds with the subject's assigned subject number. The IDS Pharmacy will call the CRC to relay the message that the IP is available for pick up. A member of the study team will deliver the hardcopy of the prescription to the IDS Pharmacy when the IP is picked up.

Example of prescription temple wording that will be used for this study is:
Cogni.Q 200mg or Placebo Capsules
Take 2 capsules by mouth in the morning (before breakfast) and 2 capsules by mouth in the evening (before dinner) as directed by the study team.
Dispense Quantity: 84 capsules
Refills: 1

Eligible subjects will be asked to fast for at least 8 hours before all of the study visits.

The following describes the study procedures:
Study visit #1: (Day 0) Subjects will come to Hershey Medical Center Clinical Research Center (CRC) prior to breakfast (fasting for 8 hours before blood draw).

- Their vitals will be taken.
- Approximately 12.5 ml (2.5 teaspoons) of blood will be collected.
- We will give subjects assigned IP for Day 0 until Day 20.
    - IDS will be responsible for dispensing IP based on a randomization table provided by study biostatistician. Therefore, subjects will be randomized (assigned by chance, like the flip of a coin) to group 1 or group 2 as defined below. They will have an equal chance to be assigned to either group. Neither the subjects nor the study staff will know which group they are in but this information is available in case of an emergency.
        - Group 1: Subjects will receive Cogni.Q for the first 21 days (day 0 to day 20). From day 21 to 34 they will take no IP (washout period). At day 35 they will start taking the placebo through day 55. From day 56 until the end of the study (day 70) they will not take any IP.
        - Group 2: Subjects will receive placebo for the first 21 days (day 0 to day 20). For days 21 to 34 they will take no IP (washout period). At day 35 they will start taking the placebo through day 55. From day 56 until the end of the study (day 70) they will not take any more IP.
- Subjects will take 2 IP capsules with water before going home at CRC and 2 more in the evening time (before dinner) for Day 0.
    - Subjects will be asked to keep a medication diary (See Supporting Materials) in which they will write the time of IP capsules taken throughout the study, and anything they experience different than normal day.
- **Study visit #2**: (Day 1) Subjects will need to come back to CRC the next morning for fasting blood draw (12.5 ml, 2.5 teaspoons). Their vital signs (blood pressure and heart rate) will be evaluated, and subjects will report any side effects/issues experienced with IP. They will swallow that day's assigned IP capsules in CRC for the morning and be discharged. They will continue with the rest of the dosing regimen, that is, subjects will be taking 2 IP capsules in the evening for that day before dinner.
- After Study visit #2 (Day 1), subjects will continue taking the IP capsules, that is 2 IP capsules before breakfast and 2 IP capsules before dinner, until the morning of day 21 which is **study visit #3.**
- **Study visit #3:** On Day 21, subjects will return to CRC, fasted, without taking any IP capsules. Subjects will return any unused IP capsules at this visit. Their vitals will be assessed and recorded, and will be asked questions regarding any missed doses, reason for missed dose(s), and any side effects experienced. 2.5 teaspoons of blood will be drawn (12.5 ml).
    - From Day 21 through Day 34, they will not take any IP capsules. This is the "*washout*" period.
- **Study visit #4:** On Day 35, subjects will return to CRC, fasted (before breakfast). Their vitals will be assessed and recorded, and will be asked questions regarding any side effects experienced during the washout period. 2.5 teaspoons of blood will be drawn (12.5 ml). Subjects will be given a refill of IP capsules. i.e. if in Group 1, a refill for placebo and if in Group 2, a refill for Cogni.Q. They will then take 2 of the assigned IP capsules and depart the clinic. They will take the other two IP capsules in the evening before dinner.
- **Study visit #5:** Day 36, subjects will return to CRC, fasted, without taking any medication. Their vitals will be assessed and recorded, and will be asked questions regarding the IP capsules, and any side effects experienced. 2.5 teaspoons of blood will be drawn (12.5 ml). They will continue to take assigned IP capsules twice (morning and evening daily) until the morning of Day 56 which is **study visit #6.**

- **<u>Study visit #6:</u>** Day 56, subjects will return to CRC, fasted. They will return any unused IP capsules at this visit. Their vitals will be assessed and recorded, and will be asked questions regarding any side effects experienced during the washout period. 2.5 teaspoons of blood will be drawn (12.5 ml). They will then enter the second washout phase.
- **<u>Study visit #7:</u>** Day 70, subjects will return to CRC, fasted, will have vitals/blood drawn (2.5 teaspoons, 12.5 ml); they will be asked about any side effects experienced during the washout period.
- A study team member will text or call weekly as a reminder to take the IP capsules.
- If subjects cannot come for a study visit on a particular day, subject needs to contact the study team so that the study team can make arrangements for the following visit. 2 days plus or minus is within acceptable study visit window, EXCEPT the 24 h visits (i.e., Day 1 and Day 36).
- Withdrawn subjects will be followed up by study staff with phone calls or electronic media within 2 weeks.
- The Table in **section 7.2.1** shows the required visits, and blood draws and the lab tests.
- Fasting blood samples will be used as follows:
    - Tube 1:
        - to analyze complete blood counts with differential (CBC w diff) to quantify neutrophils (***primary Aim 1***)
        - to quantify NK cells using immune staining and flow cytometry ***(primary Aim 2)***
        - to quantify T cells using immune staining and flow cytometry (*Secondary aim 3)*
        - Rest of the blood sample will be used to prepare plasma, which will be banked at -80^{o}C to measure trough pyranocoumarins as compliance to AGN exposure and inflammatory and immune cytokine profile (*secondary Aim 4*).
    - Tube 2:
        - We will assess NK mRNA signature by RNA-seq transcriptomics using RNA prepared from Pax Gene tube-preserved whole blood (*secondary Aim 5*). The tube will be stored at -80^{o}C until analysis time.
- The durability of the Cogni.Q supplement-induced innate immune enhancement will be assessed in the following 14 days washout period.
- Blood handling
    - Right after the blood collection, blood will be taken to the lab by a study team member on ice and processed on the same day (i.e. the primary aim studies will be performed on the day of blood collection; while plasma and Pax gene (tube 2) tubes will be banked at -80^{o}C for later use)

7.2.1 Study Schedule

Required Visit to CRC and blood draw	Time frame	CBC diff	Plasma DOH	NK and T cell flow cytometry	Pax geneRNA tube
#1, study visit, blood draw	Day 0	X	X	X	X
#2, study visit, blood draw	Day 1	X	X	X	X
#3, study visit, blood draw	Day 21	X	X	X	X
#4, study visit, blood draw	Day 35	X	X	X	X
#5, study visit, blood draw	Day 36	X	X	X	X
#6, study visit, blood draw	Day 56	X	X	X	X
#7, study visit, blood draw	Day 70	X	X	X	X

7.3 Duration of Participation

Each subject will remain in study for the duration of 70 days / 10 weeks

7.4 Test Article(s) (Study Drug(s) and/or Study Device(s))

7.4.1 Description

The commercially available dietary supplement Cogni.Q has been tested as in our completed PK study (See **Appendix 1**). Cogni.Q is sold as dietary supplement to promote cognitive agility and support healthy brain function, which are consistent with the reported neuro-protective

activities of AGN extract [11]. Cogni.Q and placebo capsules are provided by Cogni.Q manufacturer Quality of Life Labs (Purchase, NY, USA) (See letter of support, **Appendix 2**).

7.4.2 Treatment Regimen

Each subject will be instructed to take two capsules of Cogni.Q 200mg/Placebo by mouth twice daily for 21 days (Day 0 through Day 20) followed by a 14-day washout (day 21 through Day 34) and crossover to a second, 21-day treatment regimen (Day 35 through day 56). If they are assigned to group 1, participants will receive Cogni.Q 200mg on days 0 through 20 and a crossover to receive placebo on Days 21 through 34. If assigned to group 2, participants will receive placebo on Day 0 through 20 and crossover to receive Cogni.Q on Day 21 through 34.

7.4.3 Method for Assigning Subject to Treatment Groups

The statistician will generate a randomization table and provide to IDS.

7.4.4 Subject Compliance Monitoring

Study team members will text or give a reminder call to each subject to take the capsules weekly. On Day 21 and Day 56, study team will do a pill count for each subject of remaining number of capsules (to ensure that treatment regime was followed). Furthermore, plasma concentration of pyranocoumarins will be determined as compliance to AGN exposure.

7.4.5 Blinding of the Test Article

Cogni.Q and Placebo will be supplied to IDS at Penn State Hershey. IDS will be responsible for the storage, blinding, and dispensing of study medications.

7.4.6 Receiving, Storage, Dispensing and Return

7.4.6.1 Receipt of Test Article

Quality of Life will ship the Cogni.Q and Placebo capsules (cellulose) directly to IDS Pharmacy at Penn State Hershey Medical Center. An IDS staff member will inventory the shipment using the shipping invoice, notating lot number, expiration date, breakage, and total quantities. The shipment will be recorded on the study-specific drug accountability record.

7.4.6.2 Storage

The IDS Pharmacy will store the investigational product in the drug storage room within the IDS Pharmacy under the manufacturer's recommended storage conditions (room temperature). IDS Pharmacy utilizes a continuous, wireless, electronic temperature monitoring system. Temperature readings are monitored continuously and as long as no excursions occur, a recording is made every hour. Room temperature set-points are 20-25^{o}C and the system is programmed to pre-alarm if the temperature reaches 21^{o}C or 24^{o}C. When temperatures reach a pre-alarm level, the system begins contacting the pharmacy personnel via phone and pager, 24 hours a day, 7 days a week. If the area reaches an alarm level, temperatures are recorded every 5 minutes or until the area is back within the acceptable range, whichever occurs first. The system continually calls and pages until someone acts on the alarm.
STORAGE/SECURITY: Access to the IDS Pharmacy (PG200) is limited to pharmacy personnel with badge swipe access and the drug storage room(PG200A) is locked with a key. The investigational product will be stored in a bin within the drug storage room and the bin will be clearly labeled with the name of the investigational product, the PI's name, and the IRB number to clearly differentiate the investigational product from other medications that are stored in the same area.

7.4.6.3 Preparation and Dispensing

IDS will maintain a bulk supply of Cogni.Q and a bulk supply of placebo for Cogni.Q capsules. The inventory for each supply will be tracked on corresponding drug accountability records.

IDS Pharmacy will receive a patient-specific prescription for the study material (i.e., Group 1 or Group 2, take 2 capsules by mouth twice daily as directed by the study team, Dispense Quantity: 84 capsules Refills: 1). NOTE: The IDS pharmacy will only dispense investigational products after receipt of the documents listed to ensure that the patient signed consent and is enrolled in the study.

RANDOMIZATION: A randomization list will be provided to IDS that includes subject numbers and a corresponding treatment regimen (Cogni.Q 1st /placebo 2nd or placebo 1st/Cogni.Q 2nd) so that they know what to dispense to each participant.

To ensure that the blind is maintained, the investigational product name will appear as "IDS Cogni.Q 200 mg or Placebo Capsules" on the pharmacy label.

After the IDS pharmacy receives the required documentation, the IDS pharmacy staff will prepare 84 capsules (21-day supply) of the product that a subject was randomized to. A subject-specific pharmacy label will be generated and affixed to the prescription vial.

A refill will be processed for the second dispensing after the washout period. No new prescription is required at this time because a refill was added to the initial prescription. The IDS Pharmacy staff will ensure that the randomization module is followed and the correct medication is dispensed for the second treatment period.

7.4.6.4 Return or Destruction of the Test Article

The participant will return their medication vial at the visits following their 21-day treatment periods. The participant return will be documented on the corresponding drug accountability record and the empty vial and any remaining capsules will be destroyed on-site as per the IDS Pharmacy's destruction policy.

7.4.6.5 Prior and Concomitant Therapy

During the course of the study, prescription medicine and any medicine containing AGN are not permitted. At any point if a subject has to take prescription medication then they will be off the study.

8.0 Subject Numbers and Statistical Plan

8.1 Number of Subjects

40 healthy men

8.2 Sample size determination

We believe 20 participants in each group per arm in this 2 X 2 crossover design will allow us to detect an effect size of **0.75** (which is approximately equal to a difference of 920 in neutrophil mean count, assuming within-group common standard deviation is 1200, Table 2) between the two treatment arms with at least 81% power.

8.3 Statistical methods

Descriptive statistics and bivariate tests will be used to compare distributional properties of all measured variables within and between the Cogni.Q and placebo arms. The main outcome variables are

the neutrophil and NK count, which will be measured repeatedly. Linear mixed models will be used to analyze the study outcomes. More specifically, we will evaluate overall difference between the Cogni.Q and placebo arms (group effect), linear/nonlinear change over time (time effect), and group difference in this change (group-by-time interaction), separately for the pre- and post-crossover periods. We will then estimate the same model parameters but for the two crossover periods combined while controlling for potential carryover effect (i.e., group-by-period interaction). Our two-period crossover models will include the pre-crossover's baseline value (on Day 0) as a covariate common to the subsequent observations in both periods, thereby controlling for any carryover effect as well as bias due to difference at the first baseline. The outcome variables may be transformed to make sure the underlying statistical assumptions are satisfied. Other outcome variables including NK and neutrophil function assays will be analyzed in a similar statistical fashion. NK and neutrophil parameters will be examined for association for possible interaction/crosstalk. All analyses will be done using SAS version 9.4 or higher. The statistical significance level to be used is 0.05.

9.0 Confidentiality, Privacy and Data Management

9.1 Confidentiality

See the Research Data Plan Review Form

9.1.1 Identifiers associated with data and/or specimens

See the Research Data Plan Review Form

9.1.1.1 Use of Codes, Master List

See the Research Data Plan Review Form

9.1.2 Storage of Data and/or Specimens

See the Research Data Plan Review Form

9.1.3 Access to Data and/or Specimens

See the Research Data Plan Review Form

9.1.4 Transferring Data and/or Specimens

See the Research Data Plan Review Form

9.2 Subject Privacy

See the Research Data Plan Review Form

10.0 Data and Safety Monitoring Plan

10.1 Periodic evaluation of data

The frequency of data review for this study differs according to the type of data and can be summarized in the following table (Table 10.1.1)

Table 10.1.1

Data type	Frequency of review	Reviewer
Subject accrual (including compliance with protocol enrollment criteria)	Quarterly	PI, Independent Monitor
Status of all enrolled subjects, as of date of reporting	Quarterly	PI, Independent Monitor
Adherence data regarding study visits and intervention	Quarterly	PI, Independent Monitor
AEs and rates (including out-of-range lab values)	Quarterly	Study physicians, PI, Independent Monitor
SAEs	Per occurrence	Study Physicians, PI, Independent Monitor, IRB

- Independent Monitor: Andrea Manni, MD
- Study Physicians: Diane M. Hershock, MD, Ph. D, Joseph Drabick, MD and Rebecca Phaeton, MD

10.2 Data that are reviewed

Refer to Table 10.1.1 regarding who will be reviewing the data.

10.3 Method of collection of safety information

Subjects will be called weekly during the period when Cogni.Q or Placebo is given (Day 1 to Day 21 and Day 35 to 56) and reminded to take their capsules. They will be asked if they have experienced any of the expected AE (Anorexia, Nausea and Dyspepsia) or any unexpected AE that they have recorded in the medication diary.

If yes, study team personnel will inform the study physician(s) of the AE report. Study physician will contact the subject to assess the severity of AE and its relatedness to the intervention and record the data in Table 10.3.1. The physician(s) will take appropriate action of the care of the subject and make a decision in consultation with PI to whether keep the subject on the trial or withdraw.

Following table will be used to report and track any specific symptoms:

Table 10.3.1 AE reporting and tracking

Pt Identifier	AE Onset	AE End	Severity	SAE? (Y/N)	Related-ness	Action Taken	Outcome	Comments
Subj001	05/01/2019		1		2	1	4	Subject injured broke his hand
Subj002	06/26/2019		2		2	1	1	
Subj003	03/04/2019		3		0	4	4	Subj broke leg while skiing; withdrew from study

Severity of AE:
1 = Mild
2 = Moderate
3 = Severe
4 = Life threatening or disabling

Relatedness to Intervention:
0 = Definitely unrelated
1 = Unlikely
2 = Possibly related
3 = Probably related
4 = Definitely related

Action Taken:
0 = None
1 = Medical intervention (specify in comments)
2 = Hospitalization
3 = Intervention discontinued
4 = Other

Outcome:
1 = Resolved
2 = Recovered with minor sequelae
3 = Recovered with major sequelae
4 = Continuing treatment
5 = Condition worsening
6 = Patient death

Following table will be used to track subject status:

Table 10.3.2 Subject status

Pt Identifier	Date Enrolled	Date Completed Study	Study Status	Reason for Withdrawal
Subj001	03/02/2019	N/A	A	
Subj002	04/26/2019	9/20/2019	C	
Subj003	08/04/2019	N/A	W	Injury unrelated to intervention

Status:
A = Active
C = Completed
W = Withdrew
L = Lost to follow up

Following table will be used to keep track of the Frequency of specific symptom:

Table 10.3.3 Frequency of specific Symptoms

Symptoms	N%
Anorexia	
Nausea	
Dyspepsia	
Other	

10.4 Frequency of data collection

Data related to subject's safety will begin the day they are enrolled in the trial. Safety related information will be collect during the reminder calls, and by going over the medication diary maintained by the subject during their visit. Hence, the data related to subject's safety will be collected weekly and during their study visits to CRC.

10.5 Individuals reviewing the data

Refer to 10.1.1

10.6 Frequency of review of cumulative data

The PI, study biostatistician, study physicians and co-investigators will review the cumulative data. Cumulative data analysis will be done at the end of the trial (after the last subject has successfully completed the trial), since this the study subject number is less hence an interim analysis is not required.

10.7 Statistical tests

Study biostatistician will perform tests as described in 8.3. We do not see a need to perform statistics to determine safety because any occurrence will be evaluated immediately on a case-by-case basis.

10.8 Suspension of research

If any unexpected serious adverse events (SAE) occur, the research will be suspended and reports will be submitted to IRB for review and other regulatory office (FDA) as appropriate.

11.0 Risks

Physical health Risk assessment:

- Risk related to fasting:
    - Fasting for up to 8 hours could cause dizziness, headache, stomach discomfort, or fainting.
- Risk related to blood draw:
    - Blood draw needle pricks will inflict temporary superficial pain. Venipuncture might occasionally lead to localized hemorrhage and bruises at site of needle insertion. Our CRC experienced research nurses are highly trained and experienced which greatly reduces these risks. Occasionally, a subject may experience fainting spell during the blood draw. The CRC nurses are trained to respond to such situations. Subjects will be placed in a head-down position until consciousness is regained. Subjects will be monitored and given juice and/or light snacks until they are able to maintain blood pressure upon assuming an upright posture.
- Consumption of placebo vs Cogni.Q capsules:
    - As for the consumption of placebo vs. Cogni.Q capsules, we do not anticipate any more frequent or greater risks to the subject than the risks ordinarily encountered in daily life by taking a dietary supplement. Reported side effects for Cogni. Q include anorexia (lack of appetite), nausea and indigestion [12].
- Psychological, financial, legal risk assessment:
    - We do not anticipate any increased risk in these categories by participating in this study. Psychological reward might be a positive factor of participating in our study as most people derive pleasure by knowing study results may benefit themselves and many others. Financial loss due to missed work is partially mitigated by our subject remuneration plan ($25 per visit).
    - Loss of confidentiality is a potential risk when conducting human subject research and hence, all efforts will be made by the study team to ensure that loss of confidentiality does not occur.

12.0 Potential Benefits to Subjects and Others

12.1 Potential Benefits to Subjects

Since we use a crossover design, each subject will consume placebo (no risk) and Cogni.Q (being tested for acute enhancement of two types of innate immune cells) capsules. Therefore, all subjects will be participating in both groups, and hence no subject is at a disadvantage. Furthermore, there are no known benefits to the subject from participating in this study.

12.2 Potential Benefits to Others

If the efficacy results are positive for the dietary supplement (net over placebo), the public health benefit to millions, and even billions of adults in this country and globally is self-evident. Additional studies can be designed for children and special populations (e.g., therapy-induced neutropenia patients) to assess health promotional benefit and clinical therapeutic indications.

13.0 Sharing Results with Subjects

The data of the study will not be shared with the subjects.

14.0 Subject Stipend (Compensation) and/or Travel Reimbursements

Each subject will be compensated $25 per visit completed. ($200 per subject if completed; total 8 visits, 1 screening visit and 7 study visits).

15.0 Economic Burden to Subjects

15.1 Costs

The cost of the comprehensive metabolic panel that is required for determination of eligibility will be paid by the study budget. These tests are being done for research purpose only and will not be billed to the subjects or their insurance company.

15.2 Compensation for research-related injury

It is the policy of the institution to provide neither financial compensation nor free medical treatment for research-related injury. In the event of injury resulting from this research, medical treatment is available but will be provided at the usual charge. Costs for the treatment of research-related injuries will be charged to subjects or their insurance carriers.

16.0 Resources Available

16.1 Facilities and locations

The study visits will be at the CTSI CRC at Hershey Medical Center, Hershey PA.

16.2 Feasibility of recruiting the required number of subjects

CTSI CRC healthy volunteer database will be used to solicit study subjects plus multiple advertising venues of trial participation opportunity will be made in Hershey and Harrisburg metro area, including CTSI website and CTSI community engagement outreach networks, Penn State STUDYfinder, local civic institutions and grocery supermarkets.

16.3 PI Time devoted to conducting the research

PI will devote 20% time. PI and study physicians will be discussing about the study progress and frequency of AE occurrences quarterly and on a need basis in between. PI will interact and coordinate with study team members through monthly meetings and on a need basis in between. PI will participate in data analyses and interpretations along with biostatistician, CO-PI and study team personnel. Study physician(s) will perform physical exam for subjects at screening, review their lab report and determine their eligibility for participation. They will be on call to answer subject's query on study related AE issues, and review AE-related entries from medication diaries.

16.4 Availability of medical or psychological resources

Medical or psychological resources at Penn State College of Medicine are available if subject needs to use them. However, subjects will be financially responsible for utilization of any resources.

16.5 Process for informing Study Team

Monthly in person meetings will be conducted. Weekly email updates will be sent to ensure the team stays well informed.

17.0 Other Approvals

17.1 Other Approvals from External Entities

Not applicable

17.2 Internal PSU Committee Approvals

Check all that apply:

☐ Anatomic Pathology – Hershey only – Research involves the collection of tissues or use of pathologic specimens. Upload a copy of HRP-902 - Human Tissue For Research Form on the "Supporting Documents" page in CATS IRB. This form is available in the CATS IRB Library.

☐ Animal Care and Use – All campuses – Human research involves animals and humans or the use of human tissues in animals

X Biosafety – All campuses – Research involves biohazardous materials (human biological specimens in a PSU research lab, biological toxins, carcinogens, infectious agents, recombinant viruses or DNA or gene therapy).

X Clinical Laboratories – Hershey only – Collection, processing and/or storage of extra tubes of body fluid specimens for research purposes by the Clinical Laboratories; and/or use of body fluids that had been collected for clinical purposes, but are no longer needed for clinical use. Upload a copy of HRP-901 - Human Body Fluids for Research Form on the "Supporting Documents" page in CATS IRB. This form is available in the CATS IRB Library.

X Clinical Research Center (CRC) Advisory Committee– All campuses – Research involves the use of CRC services in any way.

☐ Conflict of Interest Review – All campuses – Research has one or more of study team members indicated as having a financial interest.

☐ Radiation Safety – Hershey only – Research involves research-related radiation procedures. All research involving radiation procedures (standard of care and/or research-related) must upload a copy of HRP-903 - Radiation Review Form on the "Supporting Documents" page in CATS IRB. This form is available in the CATS IRB Library.

☐ IND/IDE Audit – All campuses – Research in which the PSU researcher holds the IND or IDE or intends to hold the IND or IDE.

X Scientific Review – Hershey only – All investigator-written research studies requiring review by the convened IRB must provide documentation of scientific review with the IRB submission. The scientific review requirement may be fulfilled by one of the following: (1) external peer-review process; (2) department/institute scientific review committee; or (3) scientific review by the Clinical Research Center Advisory committee. NOTE: Review by the Penn State Hershey Cancer Institute Scientific Review Committee is required if the study involves cancer prevention studies or cancer patients, records and/or tissues. For more information about this requirement see the IRB website at: http://www.pennstatehershey.org/web/irb/home/resources/investigator

18.0 Multi-Site Research

Not applicable

18.1 Communication Plans

Not applicable

18.2 Data Submission and Security Plan

Not applicable

18.3 Subject Enrollment
Not applicable

18.4 Reporting of Adverse Events and New Information
Not applicable

18.5 Audit and Monitoring Plans
Not applicable

19.0 Adverse Event Reporting

19.1 Adverse Event Definitions

For drug studies, incorporate the following definitions into the below responses, as written:	
Adverse event	Any untoward medical occurrence associated with the use of the drug in humans, whether or not considered drug related
Adverse reaction	Any adverse event caused by a drug
Suspected adverse reaction	Any adverse event for which there is a reasonable possibility that the drug caused the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than "adverse reaction". • *Reasonable possibility.* For the purpose of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event.
Serious adverse event or Serious suspected adverse reaction	Serious adverse event or Serious suspected adverse reaction: An adverse event or suspected adverse reaction that in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.
Life-threatening adverse event or life-threatening suspected adverse reaction	An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the Investigator (i.e., the study site principal investigator) or Sponsor, its occurrence places the patient or research subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that had it occurred in a more severe form, might have caused death.
Unexpected adverse event or Unexpected suspected adverse reaction.	An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure, general investigational plan, clinical protocol, or elsewhere in the current IND application; or is not listed at the specificity or severity that has been previously observed and/or specified.

For device studies, incorporate the following definitions into the below responses, as written:	
Unanticipated adverse device effect	Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or IDE application (including a supplementary plan or application), or any other unanticipated serious problem associated with a

	device that relates to the rights, safety, or welfare of subjects.

19.2 Recording of Adverse Events

Research subjects will be routinely questioned about adverse events at study visits and during reminder calls for taking their capsules.

All adverse events (serious or non-serious) and abnormal test findings observed or reported to study team believed to be associated with the study capsules will be followed until the event (or its sequelae) or the abnormal test finding resolves or stabilizes at a level acceptable to the investigator.

Based on our prior clinical study experience (PK study) and the discussion about potential risks above (not higher than that of average life), we believe the monitoring plan involving PI/Study Physicians, independent monitor and Institutional Review Board (IRB) will be sufficient. Study physicians will be on-call and consulted to delineate whether an adverse event is study-related. Any reportable adverse effect will be timely reported to IRB-Hershey and other authorities including FDA (if warranted).

To minimize the possibility of minor and reversible side effects, presumably due to psychological anxiety, we will only recruit healthy participants (normal liver and kidney functions) and exclude subjects with cancer, and major cardiovascular diseases. In addition, we will closely monitor any possible side effect during the whole study section.

If emergency care is necessary during the study, our Study physician will be consulted. If the Study physician(s) determines emergency care is required, the subject will be referred/admitted to Hershey Medical Center where our Study Physicians has admitting privileges. The subject may be required to remain in the hospital for a period until the symptoms clear. Study physicians and PI will determine whether or not the event was related to the study. Study personnel will also document and report the adverse effects including but not limited to the following symptoms during the study to the IRB:

- anorexia (lack of appetite), nausea and indigestion [12].

19.3 Causality and Severity Assessments

The study physician will promptly review documented adverse events and abnormal test findings to determine 1) if the abnormal test finding should be classified as an adverse event; 2) if there is a reasonable possibility that the adverse event was caused by the study drug(s) or device(s); and 3) if the adverse event meets the criteria for a serious adverse event.

If the final determination of causality is “unknown and of questionable relationship to the study drug(s) or device(s)”, the adverse event will be classified as associated with the use of the study drug(s) or device(s) for reporting purposes. If the final determination of causality is “unknown but not related to the study drug(s) or device(s)”, this determination and the rationale for the determination will be documented in the respective subject’s case history.

19.4 Reporting of Adverse Reactions and Unanticipated Problems to the FDA

19.4.1 Written IND/IDE Safety Reports

Not applicable

19.4.2 Telephoned IND Safety Reports – Fatal or Life-threatening Suspected Adverse Reactions

Not applicable

19.5 Reporting Adverse Reactions and Unanticipated Problems to the Responsible IRB

In accordance with applicable policies of The Pennsylvania State University Institutional Review Board (IRB), the investigator will report, to the IRB, any observed or reported harm (adverse event) experienced by a subject or other individual, which in the opinion of the investigator is determined to be

(1) unexpected; and (2) probably related to the research procedures. Harms (adverse events) will be submitted to the IRB in accordance with the IRB policies and procedures.

19.6 Unblinding Procedures

Unblinding will occur at the end of the trial, upon PI's written request to IDS. In an event of AE, PI will have to give a written request to IDS for unblinding the subject, in order to figure out the treatment regime. AE will be reported to IRB. Anytime a person is unblinded and the cause will be reported to IRB by the PI.

19.7 Stopping Rules

Stopping Criteria:

- Any SAE that is considered life threatening, requires hospitalization or results in a disability/incapacitation.
- Any sickness that requires prescription medication.
- CBC: WBC counts over normal upper limit by 2 fold (i.e., 21,000 cells/μL) and for 2 consecutive weeks without obvious source of infection or inflammation (3,500 to 10,500 cells/μL).

20.0 Study Monitoring, Auditing and Inspecting

20.1 Study Monitoring Plan

20.1.1 Quality Assurance and Quality Control

Not applicable

20.1.2 Safety Monitoring

Not applicable

21.0 Future Undetermined Research: Data and Specimen Banking

21.1 Data and/or specimens being stored

RedCap will be used for data storage. PaxGene tubes and plasma will be stored for future use.

21.2 Location of storage

Plasma, and blood in PAX gene tubes and the extracted RNA will be stored at -80 C freezers, and the isolated PBMC will be stored in liquid nitrogen tanks at C6813 of the BMR (Immune Correlatives Lab) under lock and key with authorized access only.

21.3 Duration of storage

Data and specimen will be stored for maximum up to 10 years from the completion of the trial.

21.4 Access to data and/or specimens

Access to the data on file will be limited to PI and trial coordinator. Anyone other than PI and trial coordinator will have to notify PI and get his written permission for accessing the data.

21.5 Procedures to release data or specimens

In order to request release of any data, a written permission for data release must be obtained from the PI.

21.6 Process for returning results

Any data or results obtained from the study finding must be sent to PI, by hand delivery or secure mail.

22.0 References

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