# Safety, Feasibility and Efficacy of Sulforaphane (Avmacol Extra Strength) in Chronic Kidney Disease – Randomized, Double-blind, Placebo-controlled Trial

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Protocol

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# HEROES Study
## **H**ealth **E**ffects (**R**enal) **O**f **E**xtra **S**trength Avmacol

**Safety, Feasibility and Efficacy of Sulforaphane (Avmacol Extra Strength) in Chronic Kidney Disease: Phase II – Randomized, Double-blind, Placebo-controlled Trial**
**Principal Investigator – Thu H. Le, MD**

## 1. PURPOSE OF STUDY

We hypothesize that daily intake of sulforaphane from broccoli extract in the form of Avmacol Extra Strength (ES) can decrease kidney disease progression rate and decrease markers of oxidative stress and inflammation in Chronic Kidney Disease (CKD) patients. We will test our hypothesis in a randomized, double-blind, placebo-controlled Phase 2 clinical trial. This proposed study has been funded by National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), R01 DK128677.

We will test the safety and efficacy of Avmacol ES in CKD patients. After having established a safe dose of 4 tablets taken together daily in CKD Stages 3 – 4 in our pharmacokinetic (PK) phase (IRB protocol study 6759), we will enroll 88 patients from the Kidney Clinic in AC3, Highland Hospital, Brockport, St. James, and FF Thompson who have CKD stages 3-4 who will be randomized to Avmacol ES or placebo in a 1:1 ratio in a double-blind manner. There is a deadline for enrolling until Nov. 15, 2024, and completing study procedures by April 30, 2025, as the PI is transferring to University of California, Irvine (UCI).

## 2. BACKGROUND AND RATIONALE

In the United States (U.S.), the prevalence of CKD in adults is ~14%. The mainstay of therapy for CKD are angiotensin-converting enzyme inhibitors (ACE-Is) or angiotensin receptor blockers (ARBs), but many CKD patients still progress to End Stages Kidney Disease (ESKD) – the ultimate in failed prevention. The prevalence of ESKD is ~ 700,000, and is projected to increase to between 971,000 – 1,259,000 patients by 2030[1].

Increased oxidative stress is a major molecular underpinning of CKD progression. In humans, a common deletion variant of the glutathione-S-transferase μ-1 (*GSTM1*) gene, the *GSTM1* null allele (*GSTM1(0)*), results in decreased GSTM1 enzymatic activity and is associated with higher levels of oxidative stress. GSTM1 belongs to the superfamily of GSTs that are phase II antioxidant enzymes and are regulated by nuclear factor erythroid 2-related factor 2 (Nrf2). Our laboratory made the discovery that the highly prevalent *GSTM1(0)* is associated with more rapid CKD progression in the African American Study of Kidney Disease (AASK) trial participants[2]. This association has been replicated in the Atherosclerosis Risk in Communities (ARIC) study[3] and multiple other studies[4-8] . In experimental mouse models of CKD or hypertension, we found *Gstm1* knockout (KO) mice have increased renal oxidative stress, inflammation, and kidney injury, compared to wild-type (WT) littermate controls[9].

Cruciferous vegetables in general, and broccoli in particular, are rich in glucoraphanin, a precursor of sulforaphane (SFN) that has been shown to have protective effects against oxidative damage through activation of Nrf2. We found that dietary supplementation of sulforaphane-rich broccoli powder ameliorated kidney disease only in *Gstm1* KO mice. Similarly, in the ARIC study of nearly 11,000 patients, high intake of cruciferous vegetables was associated with lower risks of kidney failure, with stronger effects in those homozygous for the null allele (*GSTM1(0/0)*). Our published paper reporting these findings[9] was selected for news release based on "its overall excellence in furthering the field of nephrology", and was highlighted in several news outlets, including Science Daily and Reuters Health.

SFN is currently in clinical trials for cancer of the breast, lung, and prostate, as well as autism and schizophrenia (Clinicaltrials.gov). However, very few studies have assessed the effect of SFN in kidney

disease in pre-clinical experimental models; and, to the best of our knowledge, no clinical study has been performed to assess the primary outcomes of SFN in CKD that we have included in our study. Our overarching objective is to test the safety, feasibility and efficacy of SFN in delaying CKD progression, and, if so, whether its effect is dependent on *GSTM1* genotype.

Positive results in our study will also reinforce and establish further mechanistic evidence for future research on the effects of regulation of the Nrf2-GSTM1 pathway on kidney function.

Our pharmacokinetic (PK) study showed that results from a dose of Avmacol ES 2 tablets daily were highly variable and did not achieve sufficient plasma level of sulforaphane consistently. The 6 tablets daily dose was discontinued early in the study, after consultation with the Data Safety Monitoring Committee (DSMB), due to undesirable PK profile and significant gastrointestinal side effects, raising concerns for safety and tolerability if taken for a long duration. Given the favorable side effect profile of 4 tablets daily, and the more consistent result of PK profile that is similar to published studies, we have chosen 4 tablets daily for the randomized phase of our study.

## 3. ADMINISTRATIVE ORGANIZATION

This is a multi-site collaborative study funded by the National Institutes of Health (NIH). The Nephrology Division at Strong Memorial Hospital (SMH) will conduct the study to enroll patients. Subjects will be recruited from the Kidney Clinic in AC-3, located at SMH, Highland Hospital, Brockport, St. James, and FF Thompson. The University of Virginia (UVA) will serve as a data analysis participating site (see Section #17). URMC will securely transfer de-identified data to UVA for analysis and shared via REDCap.

## 4. STUDY DESIGN

**Aim 1. To test whether Avmacol ES will be safe and well tolerated in CKD patients.**

In the PK phase (IRB protocol study 6759), we established that 4 tablets of Avamacol ES is an appropriate and tolerable dose for CKD Stages 3-4 patients. We will enroll 88 patients from the Kidney Clinic in AC3, Highland Hospital, Brockport, St. James, and FF Thompson who have CKD stages 3-4 and a steady decline in estimated glomerular filtration rate (eGFR) $\geq$ 3 mL/min/$m^2$/year in the previous 12 months despite receiving standard of care. Patients will be randomized in a 1:1 Avmacol ES:placebo ratio. They will be given oral Avmacol or placebo for 6 months. Any side effects or adverse effects will be documented, and compliance to the study treatment will be monitored by tablet count at study visits. A comprehensive metabolic panel will be obtained as part of standard of care at each visit to monitor for any potential metabolic, renal or liver toxicity. The effect of *GSTM1* genotype on tolerability of Avmacol ES will be determined.

**Aim 2. To test whether Avmacol ES will improve clinical and biochemical parameters.**

At the baseline visit (month 0) and each subsequent clinic visits (typically 3 - 4 month intervals, blood pressure will be recorded, and a comprehensive metabolic profile, and urinary albumin and protein/creatinine ratio will be obtained as standard of care for patients with CKD Stages 3-4. In addition, at the baseline visit, the 1$^{st}$ month visit, and the two subsequent visits (3-4 months intervals), research lab work including markers of oxidative stress and inflammation, and podocyte damage (plasma and urine 8-isoprostane and plasma interleukin-6, and urine nephrin respectively), will also be assessed and compared between groups and to baseline within groups. Peripheral blood mononuclear cells (PBMCs) will also be obtained for analysis of mRNA levels of cytoprotective enzymes. This pilot study will allow us to begin to explore the effect of Avmacol ES on biomarkers of oxidative stress and inflammation. We will also explore the effect of Avmacol on the rate of decline of eGFR and compare between groups, and to the previous 6 months within group. The effect of *GSTM1* genotype on clinical and biochemical response will also be

assessed.

### 4.1 SUBJECT POPULATION

We will enroll 88 adult patients followed in the Kidney Clinic in AC-3, Highland Hospital, Brockport, St. James, and FF Thompson with chronic kidney disease. Evaluable subjects who withdraw from the study will be replaced by subjects with CKD stage 3 or 4 to meet the enrollment goal. We will recruit patients of any gender, race, and socioeconomic status.
Enrollment will conclude by November 15, 2024 to complete subjects' 6-month timeline by April 30, 2025. At that point the PI is transferring out of URMC.

**Vulnerable Subjects:** We do not plan to include vulnerable subjects such as children, pregnant women, fetuses, decisional impaired adults, or prisoners. Employees (physicians/fellows) will be assured that taking part in research is not a part of their duties, and refusing to participate in the study will not affect their job. To ensure their autonomy, the research coordinators or the PI who is not the patient's nephrologist will consent them.

### 4.2 STUDY INTERVENTIONS

Study Drug: Avmacol Extra Strength (ES) tablets
IND # 158689
IND Holder: Thu H. Le, MD

The study will be registered on ClinicalTrials.gov. For this double-blind and randomized, placebo-controlled trial, approximately half of the subjects will be randomized to either 4 tablets of Avmacol ES, or 4 tablets of placebo daily for 6 months. To adjust to the supplements, subjects will start the study at a dose of 2 tablets once daily for a week and then 4 tablets once daily after that for a total study period of 6 months. Nutramax will provide the tablets of both active supplement and matched placebo in bottles shipped directly to URMC Clinical Research Pharmacy Investigational Drug Services (IDS) to store, monitor, and dispense.
If the subjects experience side effects at a dose of 4 tablets together daily, they will be asked to stop taking the supplements for a week and resume at a dose of 3 tablets together daily. If they experience side effects at this dose, this will be asked to stop taking the supplements for a week again and resume at a dose of 2 tablets together daily. If they still experience side effects, they will be withdrawn from the study for safety. In case of a health emergency, the subject's healthcare provider and/or nephrologist will be unblinded to the study treatment (supplement versus placebo) by paging the IDS office.

## 5. INCLUSION AND EXCLUSION CRITERIA

**Inclusion Criteria:**

- Age $\geq$ 18 years and $\leq$ 80 years
- Estimated glomerular filtration rate (eGFR) $\geq$ 20 and < 60 mL/min/1.73m$^2$ and a decline in eGFR of $\geq$ 3 ml/min/1.73m$^2$ /year in the previous 12 $\pm$ 2 months
- Able to provide consent
- Able to swallow Avmacol ES or placebo tablets

**Exclusion Criteria:**

- Significant co-morbid conditions with life expectancy of < 1 year
- Serum potassium of > 5.5 mEq/L at screening
- New York Heart Association Class 3 or 4 heart failure symptoms, known Ejection Fraction (EF) $\leq$

30% or hospital admission for heart failure within the past 3 months
- Factors judged to limit adherence to interventions based on appointment attendance and medication treatment compliance; PI will make this determination.
- Current participation in another medical intervention study
- Known to be pregnant or planning to become pregnant or currently breastfeeding; determined by self-report and medical record history. Before dispensing the study supplements at the baseline visit, and repeated thereafter at every study visit (~month 1 and ~ every 3-4 months thereafter), a urine pregnancy test will be completed for individuals of child bearing potential, unless they have had a hysterectomy, or they are over 50 years of age and their last menstrual cycle was over two years ago.
- Cognitive impairment as determined by the patient's provider. The study coordinators will send an in-basket message to the patient's provider to determine whether they are able to provide informed consent. This will also be assessed if the patients are approached in-person after their kidney clinic appointment. Lastly, as is done for all study participants, comprehension will be tested at the time of obtaining informed consent by asking key questions about the study.
- On anticoagulants or immunosuppression
- Under treatment for cancer
- Delayed gastric emptying or similar GI conditions

Non-English-speaking individuals are excluded in this randomized phase of the study because the lack of English proficiency will affect a subject's ability to report problems or adverse events. If a patient cannot read, the consent form will be read to them by the research coordinator.

## 6. RECRUITMENT METHODS

Once eligible patients are identified, our research coordinator will reach out to each provider to ask if they would like to introduce the study to the patient and whether we may speak with their patients about the details of the study.

**Phone/Video Call**: The research coordinator will contact eligible patients by phone to introduce the study and gauge interest. IRB approved phone scripts will be used for calls.

- If the patient is interested in participating, a follow-up phone or Zoom video call will be scheduled with the patient 7 to 10 days after the initial call and recruitment email is sent.
- On the call or after obtaining informed consent, the patient will be asked for preferred method of communication: Call, Text, or Email.
- To send PDFs of the IRB approved consent form and recruitment material via email for their review, the study team member will obtain their permission to use email, per the following: "*Because URMC can't control the security of email messages once we send them, we need your permission to email you. Do you want to receive the link to the consent via email?".*
- While the subject is on the phone, our research coordinator will provide the option of reviewing the consent form either by phone or via zoom. However, obtained/documented consent will occur in person as noted in Section 7.

**Email:** One recruitment email will be sent to eligible patients after the study is introduced by phone and the patient has verbally agreed to receive recruitment email communications. If they say no, we will approach them to provide the material the next time they come to clinic for a visit.

The email will include a copy of the informed consent, a study flyer or brochure, an agreed time for a follow-up call, to occur either by phone or Zoom video, for the research coordinator to review the consent form and discuss the patient's participation further. Alternatively, this will be done at the patient's next routine health visit if it is scheduled around the time of the initial phone call.

**Texting:** Doximity, a secure texting service approved at URMC, will be used for scheduling and appointment reminders.
**Mail:** If the study participant does not prefer email, the informed consent form can be mailed with a cover letter that will instruct the potential subject not to sign/date the consent form as a follow up phone or Zoom call will be made by the study coordinator to review with them. The consent will not be signed/returned via US postal; all consent will be obtained in person; refer to Section 7-Consent Process.

**Flyer & Brochure**: Study flyers/brochures will be displayed in Kidney Clinic in AC-3, Highland Hospital, Brockport, St. James, and FF Thompson clinic waiting area and restrooms. Subjects who contact the study team directly and leave a voice mail will receive a follow-up phone call by the study coordinator, per the phone script submitted for IRB approval. If they contact the study team via email, then they will include a copy of the informed consent, a study flyer or brochure, requesting a time for a phone or Zoom video call for the research coordinator to review the consent form and discuss the patient's participation further.

**Clinic Appointment:** During the subject's Kidney Clinic appointment at either SMH or Highland Hospital, the research coordinator may approach or follow-up with the subject to discuss the details of the study and give an opportunity to ask their Nephrologist related questions or concerns. Nephrologist may also initiate information on study participation.
For the patient's convenience, the study coordinators will try to schedule study visits on or around their routine health visits to the clinic.

**@Rochester:** Information about the study will be included in the @Rochester email newsletter.

**Clinical studies admin:** A QR code or link to a prescreening form will be added to the email sent out to participants on the UR Health registry.

**MyChart:** Potentially eligible subjects will receive an email or text notifying them that they have a new research opportunity available in MyChart. It can only be viewed after they log in to MyChart and visit the Research Studies page. Subjects will see a description of the study, which is included on the attached MyChart for Recruitment Request form (uploaded as part of recruitment materials). Underneath the study description on the Research Studies page, subjects will indicate whether or not they are interested in participating by clicking on the corresponding button. This will trigger a notification that is sent to the study coordinator's InBasket in eRecord.
Interested subjects will then be contacted as described in this protocol. Subjects who are not interested will not be contacted and will be removed from the pool of eligible subjects. If subjects do not indicate whether or not they are interested in participating, they may be re-sent the email notification one additional time, no sooner than one week after the first email was sent. When study accrual has been completed, outstanding research opportunities in MyChart will be rescinded.
The study coordinators will send a direct MyChart message about the study to eligible patients via MyChart.

## 7. CONSENT (ICF) PROCESS

- Informed consent will be obtained by the research coordinator or PI and documented via a signed consent form, and stored in a locked room.
- The consent will be obtained in-person when the patient arrives for their routine lab tests at SMH or routine clinic visit at the SMH Kidney Clinic (AC3) or Highland Hospital.
- To minimize coercion, the research coordinator, the PI or co-investigator who is not the patient's nephrologist, will explain to the patient the purpose of the study and go over the consent form, and give them the opportunity to ask questions. The patient will also be given the opportunity to take the consent form home with them and take as much time needed to look

over the consent form.
- If more time is requested by the patient, further follow-ups will occur over the phone or zoom to answer questions, concerns, and discuss participation.
- The research coordinator will ask the patient to summarize the study to ensure their understanding.
- The Investigator or research coordinator will give a copy of the signed consent form to the subject and store the original appropriately in a cabinet in a locked room. If the subject has given permission to use email, the signed consent form will be scanned and emailed to them.
- A check-box for consent to contact subjects for future research will be an option with consent for this study.

***Certificate of Confidentiality:***

The study is funded by the National Institute of Health, and therefore the Certificate of Confidentiality (CoC) is automatically included as part of the notice of award. Language regarding the Certificate of Confidentiality will be included in the consent form.

## 8. STUDY PROCEDURES

A total of 4 in-person visits and 3 phone call visits will occur for the Phase II study. The baseline (month 0), first-, third-, and sixth-month visits are expected to last about 1 hour and will occur at the labs at SMH, and AC3 clinic in SMH, or Kidney clinic in Highland Hospital. The second-, fourth-, and fifth-month visits are anticipated to last about 30 minutes on the phone.

A 6-month **randomized, double-blind, placebo-controlled study** with Avmacol ES will be conducted. The study will include:

1. Pre-screening
2. Informed consent and buccal swab to determine GSTM1 genotype
3. Randomization: Patients will take the medication or placebo for 6 months, and continue to receive standard of care for CKD
4. Clinic visits for blood pressure (BP) check, labs (urine, blood) at baseline (month 0), months 1, 3, and 6. Kansas City Cardiomyopathy and NIH PROMIS questionnaires administered in-person via paper or REDCap. Side effects and adverse events (AEs) assessment; gastrointestinal (GI), heart failure symptoms
5. Phone call visits and paper/REDCap questionnaires at months 2, 4, 5: Kansas City Cardiomyopathy and NIH PROMIS questionnaires will be offered via REDCap surveys or paper copies depending on participant preference. Side effects and adverse events (AEs) assessment; gastrointestinal (GI), heart failure symptoms will be monitored

Subjects will be provided a yearly calendar to mark the days they consume the tablets and will be asked to have it available with them at each visit to monitor tablet compliance. They will also be provided a reusable medication tracker called Take-n-slide. This product is provided free of cost by the company in exchange for a simple, 5-question survey from the study subjects. The survey will be conducted approx. 1 month after the study subjects start taking the tablets, and they can keep the tracker afterwards for their use.

1. Pre-Screening: Demographics and medical history will be obtained using eRecord EMR. Each week, using the inclusion and exclusion criteria, the research coordinator will screen for patients who have a scheduled appointment in our Kidney Clinic within the next 4 weeks.

- The eGFR result should be within a 60-day window at the time of screening. If eGFR testing was performed longer than 60 days, the research coordinator will reach out to the patient's provider to ask when another eGFR will be obtained as part of the patient's standard of care and will follow

up.

- Our research coordinator will use eRecord EMR to screen patients for enrollment based on inclusion and exclusion criteria listed above in section #5. Each week, the research coordinator (RC) will screen subjects scheduled to visit to Kidney Clinic within the upcoming 4 weeks for enrollment. The RC will discuss with Dr. Le, who will make the final decision for inclusion and exclusion.
- The pre-screening data for potential subjects who choose not to participate or are ineligible will be deleted.

3. Informed consent and buccal swab for GSTM1 genotyping: Buccal (cheek) swab sample will be obtained from subject: To extract DNA for *GSTM1* genotyping to assess the effect of *GSTM1* genotype on clinical and biochemical response. Genotyping will be performed as previously done[2]. Each study participant will be assigned a subject ID by the RC after obtaining informed consent. The testing supplies for the buccal swab will be labeled by subject ID only and the lab will perform the genotyping.

3. Randomization: Randomization will be performed by the Investigational Drug Service (IDS) who will be provided the patients' CKD stage. The investigators and subjects will be blinded to the randomization of the study supplement and placebo.

At the onset, the IDS will be provided the CKD stage of each study participant (Stage 3 eGFR 30 - < 60 ml/min/1.73 m2, Stage 4 ≥ 20 – 29 ml/min/1.73 m2), The URMC Clinical Research Pharmacy and IDS will assist us in the randomization and dispensing process (their efforts are included in the NIH budget). The research team, healthcare providers and participants will be blinded to treatment assignment. Based on our patient mix in our clinic, we expect ~ 60% will be Caucasian, 40% will be AA, and ~ 50% will be female in each group.

While randomization will not be determined by *GSTM1* genotype, we will explore the effect of the genotype on response to treatment. After consent, *GSTM1* genotype will be determined from DNA extracted from buccal swab at a routine clinic visit. *GSTM1* genotype is for research purpose only and will be performed in Dr. Le's laboratory using established protocol [2,10]

4. Study visits

Patients will be given three months of ES Avmacol supplements at two timepoints each: baseline (month 0 and month 3 clinic visit). On the day of consenting to be a part of the study, if participant cannot wait until the supplements are dispensed by the IDS, they will be mailed/couriered to them.

As advised by Nutramax, study participants will be asked to take ES Avmacol after a meal, but avoid drinking hot liquids such as tea, coffee for about an hour before consuming the tablets and an hour afterwards. Study participants will be asked to bring their tablet bottles back with them at the 3 month and 6 month in-person visits to track tablet compliance. Any remaining tablets will be returned to the IDS.

To ensure patient safety our research coordinator will call subjects about 2 weeks after initiation of the study to ask how they are feeling, and whether they have developed any new symptoms. If they have new symptoms, Dr. Le will reach out to the patient to assess if further evaluation is warranted.

**I.** Clinic visits at baseline (month 0), months 1, 3, and 6

Depending on availability of appointments, these visits will be conducted at:

a. The Clinical Research Center (CRC): Blood pressure (BP) check, labs (urine, blood)

OR

b. Kidney Clinic in AC3 at SMH for BP check, and SMH labs (urine, stool)

At the baseline visit (month 0), a list of medications that the participant is currently taking will be requested.

Outcomes measured:

*The Standard of Care (SOC) lab tests* refer to Comprehensive metabolic panel (CMP), urinary albumin and protein/creatinine (PC) ratio when it is performed at a time-point recommended by the patient's healthcare provider.

*The Clinical Research lab tests* refer to Comprehensive metabolic panel (CMP), urinary albumin and protein/creatinine (PC) ratio when it is performed at a time-point not recommended by the subject's healthcare provider. These tests are for the research study only.

*The Biomarker lab tests* refer to markers of oxidative stress (plasma hydrogen sulfide ($H_2S$), plasma and urine 8-isoprostane, podocyturia (urine nephrin) to assess glomerular damage, and inflammation (plasma interleukin-6 (IL-6)) will be assessed. Peripheral blood mononuclear cells will be extracted from whole blood and saved for analysis of mRNA levels of cytoprotective enzymes (NQO1, HO-1, AKR1C1, GSTM1), and heat shock proteins (HSP27 and HSP70) [11]. The plasma $H_2S$ testing will be performed on coded samples at Cedar-Sinai and there is an MTA for it.

We will perform a urine pregnancy POCT for subjects of child-bearing potential, unless subject reports having had a hysterectomy or if subject is over 50 years of age and the last menstrual cycle was two or more years ago.

Note:

1. If patient gets their labs done before their routine clinic visit, consent will be obtained at the time they come in for their labs (if SMH). If they get their routine SOC labs done at a location that is not SMH i.e. 601 elmwood avenue before their routine clinic visit, they will be asked to provide another blood and urine sample (for biomarker lab tests only) at SMH and the research study will pay for them.

2. Patients from non-SMH locations will be asked to travel to SMH as blood samples need to be processed at Dr. Le's research lab immediately after blood draw.

3. If the subject decides to have their routine clinic visits and/or SOC lab tests done at a non-SMH location, the study coordinators will collect this data from provider ordered SOC tests from EMR.

For each lab visit related to the study, the study coordinators will carry the requisition that lists the tests to be performed by the URMC Central Lab and who would be billed (patient's insurance or research study). For example, if the patient has already had their SOC tests done, the study coordinators will indicate on the requisition that they need labs (blood and urine) for the biomarker lab tests only and it is to be billed to the research study.

On UR Box, the study coordinators will keep track of whether each lab visit was paid for by the patient's health insurance or the research study.

*Questionnaires at baseline visit & each month after:*

In addition, the Global Health, NIH PROMIS, and modified Kansas City Cardiomyopathy questionnaires, will be offered either via paper and phone or as a REDCap survey. Side effects and adverse events (AEs); gastrointestinal (GI), heart failure symptoms will be assessed.

Subjects will be asked to bring the supplement package so the study team can account for the number of tablets taken at the 1st, 3rd, and 6th month visits.

**II.** Phone calls at months 2, 4, and 5: Phone call to assess for any AEs and GI and heart failure symptoms using questionnaires. Study participants will be given the option of answering the questionnaires on the phone or via a REDCap survey.

**Health Questionnaires:** Each subject will be asked to complete three health related surveys to monitor clinical status and symptoms. These are offered electronically in REDCap using an electronic device, or in paper copies and verbally answered over the phone. Copies will also be offered through mail or email. Subjects are expected to complete the general questionnaire once a month. Each form is expected to take 20 minutes to complete.

1. **General Questionnaire:** Brief questionnaire of how the subject feels, including any new symptoms and intake of cruciferous vegetables over the last month.
2. **NIH PROMIS Questionnaire:** Questionnaire for gastrointestinal symptoms.
3. **Kansas City Cardiomyopathy Questionnaire (Modified):** Questionnaire for heart failure symptoms.

**Blood Samples:** Blood collection will involve one venipuncture 20 mL blood sample obtained at baseline visit, and 1st, 3rd, and 6th month. The samples may be obtained at the Clinical Research Center, or Strong Memorial Hospital Outpatient Lab.

**Urine Samples**: Urine collection will involve one sample of a minimum 10 mL obtained at baseline visit, and 1st, 3rd, and 6th month. Samples from subjects of child-bearing potential will also be obtained for pregnancy POCT, performed by RC or Lab staff. The samples may be obtained and processed at the Clinical Research Center, or Strong Memorial Hospital Outpatient Lab or AC1.
Whole blood will be processed for plasma and serum, divided in 200 ul aliquots, and urine will be divided in 2 mL aliquots, frozen, and stored at -80ºC for later processing and analysis in Dr. Le's lab at the University of Rochester Medical Center.

**Medical Record EMR:** No research data (*GSTM1 genotype* or biomarker levels) will be included in the subject's medical record. Laboratory data that are part of the patients' clinical care will be conveyed to the patients and providers as standard of care in their EMR. For the study, we will collect demographic information, including age, sex, height, weight, race, past medical history – specifically history of hypertension, heart failure, and etiology of kidney disease, if any - and blood pressure data. While not anticipated, incidental findings that might have health consequences for the individual subject will be addressed as clinically indicated.
As this is conducted under an IND, the samples will be stored for 2 years after marketing approval or for 5 years, whichever is later. Should additional tests be deemed informative or mechanistic to enhance further understanding, we will contact the subjects while samples are still available. Samples will be destroyed after 5 years.

**Genetic Research Procedures**: As mentioned above, the *GSTM1* null genotype is quite common in human populations, with 50% Caucasians and 27% African Americans being homozygous for the *GSTM1 (0/0*) allele. Whole genomic sequencing will not occur. No genetic counseling will be provided to the subject. We will genotype *GSTM1* to assess whether it influences any biomarker-related response to Avmacol ES.

## Schedule of Assessments

| Visit Month | Window | Location | Action items for RC | Tests & Samples |
|---|---|---|---|---|
| Baseline/ Month 0 | N/A | SMH AC3/Highland Hospital | • Informed consent<br>• Buccal swab, note eGFR (CKD stage)<br>• Dispense 3-month supply of supplement<br>• Reusable medication tracker Take-n-slide and calendar | • **BP in AC3**<br>• **Blood, Urine, (Pregnancy Test)** |
| **Visit Month** | **Window** | **Location** | • **Action items for RC** | **Tests & Samples** |
| *Month 0.5* | *+2 weeks* | *Phone call* | • *Side effects and adverse events (AEs) assessment* | *None* |
| Month 1 | +/- 2 weeks | AC3 Strong + Laboratory (Separate room) | • Side effects and adverse events (AEs) assessment<br>• GI & heart failure symptoms questionnaires<br>• Review of tablet intake<br>• Pregnancy test<br>• Take-n-slide survey | • **BP in AC3**<br>• **Blood, Urine, (Pregnancy Test)** |
| *Month 2* | *+/- 2 weeks* | *Phone call* | • *Side effects and adverse events (AEs) assessment*<br>• *GI & heart failure symptoms questionnaires*<br>• *Review of tablet intake* | *None* |
| Month 3 | +/- 2 weeks | AC3 Strong + Laboratory | • Side effects and adverse events (AEs) assessment<br>• GI & heart failure symptoms questionnaires<br>• Review of tablet intake<br>• Pregnancy test<br>• **Refill 3-month supply of supplement** | • **BP in AC3**<br>• **Blood, Urine, (Pregnancy Test)** |
| *Month 4* | *+/- 2 weeks* | *Phone call* | • *Side effects and adverse events (AEs) assessment*<br>• *GI & heart failure symptoms questionnaires*<br>• *Review of tablet intake* | *None* |
| *Month 5* | *+/- 2 weeks* | *Phone Call* | • *Side effects and adverse events (AEs) assessment*<br>• *GI & heart failure symptoms questionnaires*<br>• *Review of tablet intake* | *None* |

| | | | | |
|---|---|---|---|---|
| Month 6 | +/- 2 weeks | AC3 Strong + Laboratory | • Side effects and adverse events (AEs) assessment<br>• GI & heart failure symptoms questionnaires<br>• Review of tablet intake<br>• Pregnancy test | • **BP in AC3**<br>• **Blood, Urine, (Pregnancy Test)** |

## 9. RISKS TO SUBJECTS

**Sulforaphane/Avmacol ES (Study Drug)**
There are some potential risks to participants since we are introducing a well-tolerated study drug in other clinical trials but has not been previously tested in CKD patients. We take careful precautions by first establishing an optimal dose of Avmacol ES in CKD patients using reported peak plasma concentrations in trials that were conducted in patients without kidney disease. Based on the multiple clinical trials that have been done with Avmacol or the actual sulforaphane compound in other diseases, we do not anticipate any serious risks. The most common side effects are gastrointestinal, including nausea and dyspepsia, which are ameliorated when taken after a meal.

Although not reported in any earlier trial nor in our PK phase of the study, there is the possibility of an allergic reaction to the study drug. This reaction may be mild, such as a skin rash, or more severe symptoms like swelling of the throat, low blood pressure, and shortness of breath. In rare cases, a severe reaction could cause death.

**Risks of Blood Draw**
The most common risk is pain, bleeding, or bruising at the site of the blood draw. Other risks include redness, and swelling of the vein and infection, and a rare risk of fainting.

**Risks of Buccal Swab**
The risk is minimal. There is risk of not obtaining adequate sample for DNA extraction.

**Risks of Urine Sample**
The risk is minimal. There is risk of spilling urine onto clothes.

**Risks of Pregnancy**: The effect of sulforaphane on the fetus is not known. The patient will be advised to immediately stop taking the supplements if pregnancy is suspected and notify the research team.

**Risk of Disclosure of Privacy and Confidentiality**
Other potential risks involve violations of confidentiality. Procedures for assuring confidentiality are discussed below.

**Risks to Social/Emotional Well-Being**
We do not anticipate any psychological, social or legal risks beyond those related to participation in a clinical study.

**Risks of Questionnaires**
There are no anticipated risks for subjects to complete the requested questionnaires.

Patients will be informed that declining to participate in this study will not impact their care whatsoever.

## 10. POTENTIAL BENEFITS TO SUBJECTS

Subjects may or may not benefit from being in this research study. This study is conducted to determine safety and efficacy of the supplement on markers of inflammation and oxidative stress. The potential benefit to you might be improved levels of markers of oxidative stress and inflammation, and decreased kidney disease progression rate. Positive results from this study would provide a rationale for a much larger and longer study to determine if the medication can slow down kidney disease progression.

## 11. COSTS FOR PARTICIPATION

There is no cost for participation of the study. Avmacol ES will be provided by Nutramax without cost to the patients.

- *The Standard of Care (SOC) lab tests**:*** The subject and/or their insurance company will be responsible for the costs of these tests.
- *The Clinical Research lab tests*: The research study will pay for all bills associated with these tests.
- *The Biomarker lab tests*: The research study will pay for any bills associated with these tests.

## 12. PAYMENT FOR PARTICIPATION

For this study, we will use a subject payment system called Advarra Participant Payments. The system allows three ways to provide payment. The subjects can choose: a reloadable debit card; direct deposit; or mailed paper checks.
Payment: Compensation will be divided into four payments of $25.00 at month 0 (baseline), month 1, month 3, and month 6. Subjects who are withdrawn or voluntarily withdraw from the study due to side effects will also receive the full compensation of $100.
Parking pass: Study participants will be provided a parking pass for their study visits to the SMH. Patients from non-SMH locations who travel to SMH for their labs (blood, urine) and BP checks will also be provided a parking pass. For participants with severe ambulatory challenges, an option to be reimbursed for valet parking service will be offered.
Bus pass: An option for a bus pass will be provided to study participants

## 13. SUBJECT WITHDRAWALS

1. Participants' decision to withdraw from the study for any reason
2. Development of side effects that are not resolved by dose reduction
3. Failure to adhere to the study protocol
4. Relocation resulting in inability to follow up per protocol
5. Become pregnant
6. New diagnosis of heart failure, cardiovascular event, life threatening infection, or cancer

In the event that a subject drops out the study due to reasons listed above, we will recruit an additional patient to replace.

**Return of Results**:
Once the study is completed, we will send subjects a summary of the results and what they mean. Subjects will not receive their own individual results.

## 14. CLOSE-OUT PROCEDURES

At the conclusion of the study, subjects will receive a mailed letter and phone call with a summary of results, which Arm of investigational product they took (Active or Placebo), and the ClinicalTrials.gov study NCT information. The PI's plan to transfer to UC Irvine will also be shared.

Unblinding: The IDS will reveal the randomized groups of subjects to the PI and Coordinators one day after the final subject lab visit. Lab staff will remain blinded until the assay processing is completed. Unblinding is allowed before analysis.

## 15. PRIVACY AND CONFIDENTIALITY OF SUBJECTS AND RESEARCH DATA

The research team consists of URMC clinical providers (Nephrologists) and as such have access to all of the relevant clinic records. Study data will be collected and managed using REDCap electronic data capture tools hosted at URMC. Study data will be directly entered into REDCap, a HIPAA-compliant database. Only de-identified data will be analyzed, and de-identified data will be stored in a password-protected database only available to the research team.

With regard to patient confidentiality, the risk of improper access, use, or disclosure of participant data is minimized through the following procedures:

1. All study personnel and researchers are required to sign a pledge of confidentiality, committing to safeguard the study data during its collection, analysis, and after the study is concluded.

2. Physical (paper) questionnaire forms and media containing the electronic data files will be kept in locked files with restricted access.

3. Data Key- Each subject will be assigned a study ID number which will be used to label their data/samples and linked to the subject on a separate key. Only Dr Le and the investigative team will have access to the data key and it will be stored separately from the dataset.

4. The data upon which statistical analysis will be conducted will contain participant numbers as unique identifiers in lieu of names, medical record number, or social security numbers. These data are considered identifiable, but not directly identifiable.

When data generated from this study is shared for analysis, only de-identified information will be exchanged. No protected health information will be shared with any collaborator nor used in any publications. Only aggregate results, not individual data, will be published in reports or manuscripts.

## 16. DATA / SAMPLE STORAGE FOR FUTURE USE

Samples will be collected, processed, and stored for future use in an approved -80$^0$C freezer. To minimize costs, they will be run in batches based on the number of samples that can be loaded on commercially available kits. As this is conducted under an IND, the samples will be stored for 2 years after marketing approval or for 5 years, whichever is later. Only Dr. Le and the investigative team will have access to the

stored data and samples.

The investigative team may elect to share with other researchers and/or utilize subject data and biospecimens collected during this study for future research purposes. This includes subject samples, health information, and genotype GSTM1 data. Subjects will be provided the opportunity to opt-in or opt-out of permitting the study team to utilize their data and biospecimens during the ICF process. Should subjects agree to allow the study team to utilize data and biospecimens for future research, the data and biospecimens would be used to further understand kidney disease and other related topics.

The clinical data and associated biospecimens will be identified only by the Subject ID used in the current study. Computers housing the data are secured in locked rooms when not in use. All data files and derived analytic data files are encrypted for storage and transport. Study data will be kept on password protected computers at URMC, REDCap and UR Box program. Signed consent forms will be stored in a cabinet in a locked room.

## 17. DATA AND SAFETY MONITORING PLAN

**Medical Supervision and Surveillance of Study Subjects.** The PI, Dr. Le, will be responsible for supervision of the entire study. Clinical status and symptoms will be monitored using comprehensive health screening tools, including the NIH PROMIS questionnaire for gastrointestinal symptoms, and a modified Kansas City Cardiomyopathy questionnaire for heart failure symptoms that will be relevant to both patients with a history of heart failure (NY Heart Association Class 3 and 4 heart failure symptoms are excluded) and patients without pre-existing history of heart failure.

**Data Safety Monitoring Board (DSMB).** We have already identified 3 members at URMC who are not investigators in the study to serve on the DSMB:

1. Ronnie Guillet, MD, PhD, Professor of Pediatrics. Over the course of her career, Dr. Guillet has been involved in two primary areas of research, both focused on better understanding neonatal development and injury: neonatal brain injury (neonatal seizures and hypoxic ischemic encephalopathy) and their treatment and neonatal acute kidney injury (epidemiology, contributing and ameliorating factors). These efforts and others have resulted in over 100 peer reviewed publications. She has collaborated with colleagues across the country and around the world, has been invited as a speaker to a variety of regional and national meetings, and has been asked to collaborate on a number of multicenter clinical trials. In addition, she has chaired 4 Data Safety Monitoring Committees, including 2 sponsored by NIH NINDS.

2. David Bushinsky, MD, Professor of Medicine, Pharmacology and Physiology. Dr. Bushinsky has published nearly 120 peer-reviewed articles and over 60 invited reviews, chapters and editorials focusing on disorders of divalent ion metabolism. He has conducted large clinical trials, including "The effects of the potassium-binding polymer patiromer on markers of mineral metabolism (CJASN 2019)", "Veverimer versus placebo in patients with metabolic acidosis associated with chronic kidney disease: a multicenter, randomized, double blind, controlled, phase 3 trial (Lancet 2019)", and "Long-term safety and efficacy of veverimer in patients with metabolic acidosis in chronic kidney disease: a multicenter, randomized blinded placebo-controlled, 40-week extension (Lance 2019)".

3. Dongmei Li, PhD, Associate Professor Public Health Sciences, in the Clinical and Translational Science Institute (CTSI). She has more than ten years of experience conducting statistical methodology research, teaching, mentoring public health students, and providing consulting

services for biomedical research. She is serving as Program Director of the Biomedical Data Science Certificate Program co-sponsored by CTSI and Department of Public Health Sciences. She also serves as the Biostatistics and Informatics Core Director of an FDA/NCI funded 19 million U54 center grant on flavored tobacco products. For her biostatistics methodology research, she has worked on both genetic and genomic data analysis (gene expression, SNP, DNA methylation, ChIP-seq, ATAC-seq, and proteomics) for more than ten years, and has developed new statistical analysis methods and corresponding analysis packages in R/Bioconductor for DNA methylation array and RNA-Seq data analysis. She also developed a new method and R package to control for multiple testing error rates in genomic and genetic data analysis.

The DSMB will meet at the beginning of the randomized trial, every 3 months thereafter, and at the conclusion of the trial, or sooner if there are unanticipated side effects and adverse events. The DSMB will follow the NIH National Center for Research Resources guidelines including:

1. Review subject recruitment, attrition, and minority involvement
2. Monitor safety of research participants by reviewing unblinded data for side effects and adverse events
3. Assuring compliance with requirements relating to reporting of adverse events
4. Assuring data accuracy and protocol compliance, and that any action that results in the temporary or permanent suspension of the protocol is reported to all appropriate monitoring bodies, including the IRB, and the NIH Office of Biotechnology Activities.

Should any serious adverse event occur, we will convene a meeting with the DSMB to determine whether the study should be suspended until additional review can be properly performed.

## 18. DATA ANALYSIS PLAN – To be performed at UVA

Data harmonization, management and analysis

1. Develop data management and statistical analysis plan.
2. Work with the team and CTSI at URMC closely to design a unified management system for data collection, transfer between the study site and management.
3. Data oversight and monitoring during data collection and integration.
4. Harmonize the study data elements and variables from clinical and laboratory and biochemical data
5. Validate data quality and perform cleaning process.
6. Work closely with the PI and clinical investigators to determine appropriate statistical methods and machine learning algorithms for the clinical hypotheses. Cutting-edge statistical methods and innovative data science tools will be considered or adopted.
7. Prepare the analytical datasets and perform pre-stated statistical analyses.
8. Prepare the final results for manuscript writing and submission, conference presentations and publications.

**Power calculation**: The primary outcome of interest in this study is plasma 8-isoprostane, one of the markers of oxidative stress. Based on: 1) the inverse correlation between 8-isoprostane and eGFR (Figure 1 of Cottone *et al.*[12]), 2) the average 8-isoprostane for hypertensive patients of 262 ± 137 pg/ml, 3) a range from 350 to 550 pg/ml for CKD Stages 3-4 patients, and 4) therefore the assumption that the average 8-isoprostane would be 400 ± 150 pg/ml in placebo group, we hypothesize that Avmacol ES would reduce by 25%, approximately at 300 pg/mL.

According to power calculations, the minimum number of participants that need to be recruited is 88. To address the challenges of recruiting patients with stage 4 CKD, there will not be a fixed proportion of patients with CKD stage 3 and 4 that will make up the final 88. However, an equal number of subjects

regardless of CKD stage (44 each) will be assigned by the IDS to the Avmacol ES and placebo arm of the study. **The final 88 will include the ones already enrolled.**

If study participants drop out, additional patients with CKD stage 3 or 4 will be recruited to reach this number. For example, if 8 out of 88 participants drop out, 8 more would be enrolled so the final number of enrollments would be 96.

If a CKD stage 4 patient drops out, a CKD stage 3 patient may be enrolled to make up for the drop-out to reach the total of 88 study participants. Treatment assignments will be performed by the IDS, and the study team will remain blinded.
With 44 patients in Avmacol ES group and 44 in placebo group who would complete the study, we expect to have power to detect the difference in 8-isoprostane between the two groups. The power analysis was performed using two-sample *t*-test with 5% type I error. The effect size is considered close to medium effect, which is appropriate for this pilot study as we intend to establish preliminary evidence of treatment effect of Avmacol ES. As mentioned above, *the Kidney Clinic in AC3 sees ~ 800-900 patients with CKD a month and ~ 60% of patients are in the CKD Stages 3-4 stages. Thus, in addition to Highland Hospital, we should be able to easily achieve our recruitment target.*

In the event that patients drop out the study due to relocation or loss to follow up, we will recruit additional patients. Based on the number of dropouts at 12 months into the study, we will recruit an extra number of patients commensurate with the dropout rate such that we will have the anticipated number of patients (44 in each group) who will complete the study in 24 months.

**Statistical Analysis:** The data for safety and feasibility assessment will be summarized descriptively with respect to adverse events and compliance with Avmacol ES intake. The difference between the two treatment groups will be evaluated with Chi-square test, and logistic regression if necessary. Distributions of outcome variables will be examined graphically for asymmetry and for outliers. If a lack of symmetry is noted, the variable will be transformed before analysis. The group-specific data will be summarized and reported as mean ± SD or median (interquartile range) for continuous measures and as frequency and percentage for categorical measures and their differences will be compared using two-sample *t*-tests or Chi-square test. Those baseline characteristics that are significantly different between the 2 groups will be adjusted in the subsequent analyses. Since plasma 8-isoprostane will be measured at baseline, 1, 3, and 6 months, the effect of Avmacol will be explored at each time point with *t*-test or linear regression to detect an early effect of Avmacol at 1 or 3 months and to determine if such effect will be sustained at 6 months while the patients remain on Avmacol. The effect of Avmacol on longitudinal plasma 8- isoprostane measures will be analyzed with linear mixed effects model, adjusting for potential confounding factors such as age, initial BMI, and baseline characteristics identified in the preliminary analysis. Other factors that might affect the results (such as physical activity and dietary intake) will also be examined. Similar analyses will be performed for the secondary outcomes, including plasma IL-6, urine 8-isoprostane, and urine nephrin. We will also explore the effect of Avmacol on eGFR with linear mixed effects modeling by comparing the slope of eGFR decline between groups, and to the previous 6 months within group, and the effect of *GSTM1* genotype on the response to Avmacol. Additionally, penalized regression and machine learning methods such as random forests algorithm will be considered and applied to fully evaluate the treatment effect and predictability of Avmacol and other important predictors on the outcomes.

The data for safety and feasibility assessment will be summarized descriptively with respect to adverse events and compliance with Avmacol ES intake. The group-specific data will be summarized and reported as mean ± SD for continuous measures and as frequency and percentage for categorical measures and their differences will be compared using two-sample *t*-tests or Chi-square test.

## 19. PI oversight for multi-site research

The UR PI will be responsible for the following:

A. Communication: The UR PI will notify the Site PI of the RSRB site approval and provide site approval letter as well as copies of the most current version of the study materials via email. The UR PI is responsible throughout the course of the study to provide all modified documents and IRB approval letters to UVA site PI.

B. Supervision of study activities at the site: The UR PI will conduct regular meetings with the site PI to review study activities. Meetings will occur every 3 months or sooner as soon as any issue arises by zoom. During the meeting, the UR PI will troubleshoot study-related issues, answer study-related questions, and provide verbal/written direction for all study activities. Meetings will be documented via meeting minutes in the regulatory binder.

C. Training: The UR PI will ensure that all study personnel have completed required institution-specific and protocol-specific trainings and that these trainings are documented appropriately via CITI certification. When study personnel are added/removed, the UR PI will ensure UVA submits these changes to the local IRB for institutional review and approval. If applicable, these changes will be appropriately documented on the Delegation of Responsibilities Log.

D. Reporting: The UR PI will submit required information for reporting, progress reports, reportable events, non-compliance, Data and Safety Monitoring reports and provide the site with any RSRB determinations regarding the submitted reports.

E. Site Files and Documentation: As per Policy 901 Investigator Responsibilities, all sites will maintain a regulatory file with current and accurate records of all study documentation as required by applicable regulatory requirements. These files are maintained electronically in secure BOX URMC servers and shared with study members only.

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11. Liu, H.*, et al.* Biomarker Exploration in Human Peripheral Blood Mononuclear Cells for Monitoring Sulforaphane Treatment Responses in Autism Spectrum Disorder. *Scientific reports* **10**, 5822 (2020).
12. Cottone, S.*, et al.* Endothelin-1 and F2-isoprostane relate to and predict renal dysfunction in hypertensive patients. *Nephrol Dial Transplant* **24**, 497-503 (2009).