Safety, Feasibility and Efficacy of Sulforaphane (Avmacol Extra Strength) in Chronic Kidney Disease – Phase I
Principal Investigator – Thu H. Le, MD

1. PURPOSE OF STUDY

We hypothesize that daily intake of sulforaphane (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 1/2 clinical trial in a single center, two sites, 3-year study. This proposed study was submitted as an R01 application in response to FOA PAS-20-160 that encourages submission of pilot and feasibility clinical trials that will lay the foundation for larger clinical trials related to the prevention/treatment of CKD within the mission of NIDDK. We have received a Notice of Award for this study from NIH/NIDDK.

There are two phases to our study; each will have a separate Consent Form. This proposal is only for Phase I study. Once Phase I is complete, a new study will be submitted for Phase II. Please see study design below for details:

1. Phase I: Establish pharmacokinetics of Avmacol ES in patients with CKD Stages 3-4 (first 6 months of the study). Subjects who participate in the first phase of the study may be eligible to participate in the second phase of the study.

We seek approval for the Phase I of the study which will establish the dosing for the second phase of the study.

2. BACKGROUND AND RATIONALE

In the United States (U.S.), the prevalence of chronic kidney disease (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 stage 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]. 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 [4].

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 recently published paper reporting these findings [4] 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, and cardiotoxicity from chemotherapy (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 effect of SFN in CKD. 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.

Once Phase I is completed and an appropriate dose is established, we will move to our Phase II pilot study in which patients will be randomized to the study drug Avmacol ES and placebo for 6 months. We hope that at the conclusion of the 3-year study, SFN will be demonstrated to be safe and well tolerated, with measurable pharmacodynamic actions. The results would provide a sound rationale for Phase III and a future large multicenter clinical trial to randomize CKD patients to standard care ± SFN to determine its efficacy in slowing the rate of decline of eGFR in patients with CKD Stages 3-4 and its interaction with *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.

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, and from Highland Hospital. University of Virginia will serve as a data analysis site (see Section #17). URMC will securely transfer de-identified data to UVA for analysis.

4. STUDY DESIGN

Phase 1 study that will be conducted to establish pharmacokinetics of Avmacol ES in patients with CKD Stages 3-4 and includes the following end-points:

Aim: To determine the pharmacokinetics of Avmacol ES in patients with CKD Stages 3-4 and the influence of *GSTM1* genotype on bioavailability. Nutramax has expressed willingness to provide Avmacol ES, a commercially available glucoraphanin packaged with the active enzyme myrosinase to convert glucoraphanin to SFN. Regular Strength Avmacol has been tested in 16 clinical trials, https://clinicaltrials.gov/ct2/results?cond=&term=Avmacol&cntry=&state=&city=&dist=, 9 of which are still active. We will first establish an optimal dose for CKD Stages 3 and 4 patients to achieve similar pharmacokinetic (PK) profile observed in non-CKD patients. We will also test our hypothesis that there is higher bioavailability of SFN in CKD 3-4 patients carrying the *GSTM1(0/0)* genotype compared to those carrying the active *GSTM1* allele.

4.1 STUDY INTERVENTIONS

STUDY DRUG: Avmacol Extra Strength capsules
IND # 158689
IND HOLDER: Thu H. Le, MD

For Phase I, we will only use Avmacol ES, no placebo, to determine the PK of Avmacol ES in CKD to guide the dose used for Phase II.

Nutramax Laboratories, Consumar Care, Inc., will provide Avmacol ES capsules free of charge in bottles shipped directly to University of Rochester Medical Center (URMC) Clinical Research

Pharmacy Investigational Drug Services (IDS). The IDS will store, monitor, and dispense the drug directly to subjects. Data from the study will be shared with Nutramax.

It should be noted that, compared to control, SFN at "low dose" or 0.5 μM concentration, *ex vivo*, is able to induce expression of the genes in human peripheral blood mononuclear cells (PBMCs) that encode the enzymes AKR1C1 and NQO1 that play a role in reducing radical species [5]. Higher doses of 2 μM and 5 μM of SFN induce higher expression of AKR1C1 and NQO1, as well as HO-1. Other human trials with SFN have reported higher peak plasma concentrations of 1-2 μM [6].

GSTM1 genotype will first be determined for the PK analysis to assess whether the genotype influences drug metabolism. Stratified by *GSTM1* group, patients will be assigned randomly to 2 or 4 Avmacol Extra Strength tablets once a day, taken orally with food, as depicted in **Table 1**. The groups are categorized into *GSTM1* null (*GSTM1 (0/0)* or *GSTM1* non-null (*GSTM1 (1/1)* or *(1/0)*) genotype based on the dominant model we observed in AASK and ARIC cohorts [2,3].

Table 1: Number of Avmacol Extra Strength Tablets Once a Day

GSTM1 Genotype	**2 Tablets**	**4 Tablets**
GSTM1 (1/1 or 1/0)	6	6
GSTM1 (0/0)	6	6

Between 800-900 patients are seen monthly in the URMC Kidney Clinic in AC-3 and Highland Hospital. Among these patients, ~ 60% have CKD Stage 3 or 4, and ~ 40% of the total patients are African American. The null genotype is quite common in human populations, with 50% Caucasians and 27% African Americans being homozygous for the *GSTM1 (0/0*) allele (see **Table 2** for prevalence of *GSTM1* genotypes) [7]. We saw similar prevalence of genotypes in AASK and ARIC [2-4]. Therefore, we do not anticipate difficulty identifying those with null versus non-null genotypes.

Table 2	**Prevalence**	
***GSTM1* Genotype**	**Caucasians**	**African Americans**
GSTM1 (1/1 or 1/0)	**50%**	**73%**
GSTM1 (0/0)	**50%**	**27%**

Rather than using a dose escalation approach which would take more time, we will be assessing 2 different doses simultaneously in different participants in each group to assess for the degree of variation in PK. We will choose the highest dose that achieves similar $t_{1/2}$ concentration and AUC_{0-8} of SFN levels as observed in non-CKD patients (1-2 μM) [5,6] for our safety and efficacy study in Phase II.

Toxicities and guidelines for adjustments, withdrawals, etc.

In case of any significant side effects, adverse events or unusual changes, dosage will first be halved, and if patient's signs/symptoms do not improve after 3 days, treatment will be stopped. If signs/symptoms are resolved, the treatment will be resumed at the lower dose for 7 days. On Day 7 when the patient comes in for PK study. Otherwise, the study treatment will be stopped. If symptoms recur after resuming the lower dose, Avmacol ES will be discontinued.

Reporting of Side Effects. Participants will be given instructions and contact information to report any side effects, new symptoms or worsening of preexisting symptoms to the research coordinator or their primary nephrologist (who will be made aware of the patient's participation) during work hours. After work hours, including nights and weekends, they will contact Dr. Le who is available 24 hours a day. If Dr. Le is away, the Nephrology physician on call will take the calls for Dr. Le, so that 24/7 medical coverage will be available for study participants. Any serious unexpected adverse effects will be immediately reported to the IRB, the DSMB, and the NIH Office of Biotechnology Activities.

4.2 SUBJECT POPULATION

We will enroll 24 adult patients followed in the Kidney Clinic in AC-3 with chronic kidney disease. Evaluable subjects who withdraw from the study will be replaced to meet the enrollment goal. We will recruit patients of any gender, race, and socioeconomic status.

Vulnerable Subjects: We do not plan to include vulnerable subjects such as children, pregnant women, fetuses, 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 coordinator (rather than the PI) will consent them.

5. INCLUSION AND EXCLUSION CRITERIA

Inclusion Criteria:

- Age ≥ 18 years and ≤ 80 years
- Estimated glomerular filtration rate (eGFR) ≥ 25 and < 45 mL/min/1.73m^2
- Able to provide consent
- Able to swallow Avmacol ES capsules

Exclusion Criteria:

- Significant co-morbid conditions with life expectancy of < 1 year
- Uncontrolled hypertension; an average recorded blood pressure ≥140/90 mmHg in the past 6 months
- Serum potassium of > 5.5 mEq/L at screening
- New York Heart Association Class 3 or 4 heart failure symptoms, known EF ≤ 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 study
- Known to be pregnant or planning to become pregnant or currently breastfeeding; determined by self-report and medical record history. A urine pregnancy test will be completed for individuals of childbearing potential before administering the study drug.
- History of dementia documented in the medical record
- On anticoagulants or immunosuppression
- Under treatment for cancer

Non-English-speaking individuals will be not be included in Phase I of the study, but will be included in Phase II. 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 them if we may speak with their patients for the study.

Phone/Video Call: The research coordinator will contact eligible patients by phone to introduce the study and gauge interest.

- 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.

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. 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?".* Permission will be obtained before a PDF of the IRB approved consent form and study flyer is sent via email for their review.

The email will include a copy of the informed consent; a study flyer; 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.

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.

Mail: Mailing the informed consent form with a cover letter that will instruct the potential subject not to sign/date the consent form as 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: Study flyers will be posted in Kidney Clinic in AC-3 and Highland Hospital clinic waiting area and restrooms. Subjects who contact the study team directly and leave voice mail will receive follow up phone call by the study coordinator, per the follow up recruitment phone script submitted for IRB review.

7. CONSENT PROCESS

- Informed consent will be obtained by the research coordinator and documented via a signed consent form, and stored in a cabinet in a locked room.
- The consent will be obtained in-person when the patient presents to the Kidney Clinic for their routine follow up.
- To minimize coercion, the research coordinator, and 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 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.
- 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

SCREENING PROCEDURES:
Avmacol ES has not been tested in patients with CKD. Since sulforaphane (SFN) and its metabolites are cleared by the kidney, to establish a safe dose for our study participants, for Phase I of the study, we will first perform PK analysis in CKD Stage 3B to 4 patients with eGFR between 25 – 45 mL/min/1.73m^2 who meet the inclusion criteria below. The eGFR result should be within a 2 week window at the time of screening. If eGFR testing was performed longer than 2 weeks, the research coordinator will reach out to the patient's provider to ask for repeat testing.

Our research coordinator will use eRecord EMR to screen patients for enrollment based on inclusion and exclusion criteria listed above in #5. Each week, the research coordinator (RC) will screen subjects scheduled to visit to the Kidney Clinic in the upcoming 1 to 2 weeks for enrollment. The RC will discuss with Dr. Le, who will make the final decision for inclusion and exclusion.
If eligible and after subject consent is obtained:

A total of 2 in-person study visits will occur for the Phase I study. The first visit (AC3 or Highland Hospital) is expected to last for 1 hour. The second visit will only occur at the Clinical Research Center in Strong Memorial Hospital (SMH) and will take place on day 7 of taking the study drug and is expected to last up to 9-hours to allow biospecimen collections at 5 separate intervals.

RANDOMIZATION:

As noted in Section 4.1 Study Interventions, groups will be stratified by *GSTM1* group, patients will be assigned randomly to 2 or 4 Avmacol Extra Strength tablets once a day, taken orally with food, as depicted in **Table 1 (below)**. The groups are categorized into *GSTM1* null (*GSTM1 (0/0)* or *GSTM1* non-null (*GSTM1 (1/1)* or *(1/0)*) genotype based on the dominant model we observed in AASK and ARIC cohorts. We do not anticipate difficulty obtaining the different genotype groups, as 75% of Caucasians are either *GSTM1 (1/1)* or *(1/0),* and 25 % are *GSTM1 (0/0)*. Approximately 65% of patients in our Kidney Clinic are Caucasians. In African Americans/blacks, approximately 25% are *GSTM1 (1/1),* 50% are *GSTM1 (1/0),* and 25 % are *GSTM1 (0/0)*. Approximately 30% of patients in our Kidney Clinic are African Americans. Randomization will be performed by the IDS who will be provided the patients' eGFR and GSTM1 genotypes. Since this is a PK study, the investigators will not be blinded to the randomization.

Visit 1 (1 hour) at AC3 or Highland Hospital Procedures:

Buccal Swab: A buccal (cheek) swab will be obtained from the subject to extract DNA for *GSTM1* genotyping for assignment to groups as outlined in **Table 1**. (below). Genotyping will be performed as previously done [2]. The processing of DNA and genotyping will be completed within 5 business days.

Drug Dosing: In-person at the time of their study visit, patients in each dose group will be provided 2 or 4 tablets to be taken at home once a day with food for 7 days.

Table 1: Number of Avmacol Extra Strength Tablets Once a Day

GSTM1 Genotype	**2 Tablets**	**4 Tablets**
GSTM1 (1/1 or 1/0)	6	6
GSTM1 (0/0)	6	6

NIH PROMIS Questionnaire: For each subject, clinical status and symptoms will be monitored using this questionnaire for gastrointestinal symptoms. Paper copies of this survey will be administered to the subject at Visit 1 and each form is

expected to take 20 minutes to complete. The questionnaire will be completed in-person as part of Visit 1, at home on Day 3, and returned with the next in-person visit on Day 7 (Visit 2).

Kansas City Cardiomyopathy Questionnaire (Modified): For each subject, clinical status and symptoms will be monitored using this questionnaire for heart failure symptoms. Paper copies of this survey will be provided to the subject at Visit 1 and are expected to take about 30 minutes each to complete. The questionnaire will be completed in-person as part of Visit 1, at home on Day 3, and returned with the next in-person visit on Day 7 (Visit 2).

General Questionnaire: Each patient will also complete a brief questionnaire of how they feel each day, including any new symptoms. Subjects are expected to complete the general questionnaire once each day throughout the 7-day period.

Subject Tracking Chart: For each subject, a chart will be provided to help track daily study drug intake and questionnaires. The form will also include the subject's study drug dosage and 7-day study timeline.

Visit 2/Day 7 Procedures (8-Hours) at Clinical Research Center in Strong Memorial Hospital:

On day 7, subjects will come to the Clinical Research Center at SMH, take their dose with food. This visit will last for about 8-hours in duration so samples of urine and blood samples can be obtained at scheduled time intervals. Food and drink will be provided for lunch during the 8-hour visit.

Subjects will be asked to return the drug package so the study team can account for the number of tablets taken during the 7-day period.

Blood Samples: Blood collection will involve one venipuncture 4 mL blood sample obtained at 0, 1 hour, 2, 4, and 8 hours. The samples may be obtained by a phlebotomist in the Clinical Research Center or Strong Memorial Hospital Outpatient Lab. An IV-line will be placed for the blood draws to minimize needle sticks. A total number of two collection attempts will be allowed for each time interval.

Urine Samples: Urine collection will involve one sample of a minimum of 1 mL of urine in a sterile cup obtained at 0, 1 hour, 2, 4, and 8 hours. Water will be provided between each sample collection.

Blood and urine will be obtained at 5 separate intervals listed above to determine SFN levels in plasma and urine, respectively. Whole blood, plasma, and urine at each time point will be obtained, frozen, and stored at -80^{o}C for later processing and analysis. Levels of SFN and its active metabolites will be analyzed by our Mass Spectrometry Shared Resources Laboratory (MSRL) Core at the University of Rochester Medical Center.

Medical Record EMR: No research data (*GSTM1 genotype* or blood SFN level) 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 as standard of care. 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. Research data will not be conveyed to the patients. 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 PK/drug metabolism of SFN from Avmacol.

Completion of the first phase: To ensure patient safety after they have completed or stopped the study drug, our research coordinator will call subjects after about 2 weeks 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.

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 with food.

Although not reported in any earlier trial, 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 drug 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

You might not benefit from being in this research study. Your participation will enable us to determine the best dose for the next study in patients with chronic kidney disease in which you would also be eligible to participate in.

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.

12. PAYMENT FOR PARTICIPATION

Each of the study participants will receive $100.00 in a prepaid Visa card, divided into two payments of $50.00 at Visit 1 and Visit 2, as payment for their participation and completion of the study. Subjects who are withdrawn or voluntarily withdraw from the study due to side effects will also receive the full compensation of $100.

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 patient drops out the study due to reasons listed above, we will recruit an additional patient to replace.

Once treatment is stopped for any reason, patients will continue to be followed as part of standard of care for CKD. Long-term follow up data will continue to be collected, including their blood pressure, eGFR, and proteinuria.

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. 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 which is 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.

15. 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.

16. 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 nearly 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 Phase I and at the end of Phase I which is anticipated to be completed in 6 months, and 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.

17. 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.

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.

18. 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.

19. REFERENCES

1. McCullough, K.P., Morgenstern, H., Saran, R., Herman, W.H. & Robinson, B.M. Projecting ESRD Incidence and Prevalence in the United States through 2030. *J Am Soc Nephrol* **30**, 127-135 (2019).
2. Chang, J.*, et al.* Loss of GSTM1, a NRF2 target, is associated with accelerated progression of hypertensive kidney disease in the African American Study of Kidney Disease (AASK). *Am J Physiol Renal Physiol* **304**, F348-355 (2013).
3. Tin, A.*, et al.* The Loss of GSTM1 Associates with Kidney Failure and Heart Failure. *J Am Soc Nephrol* **28**, 3345-3352 (2017).
4. Gigliotti, J.C.*, et al.* GSTM1 Deletion Exaggerates Kidney Injury in Experimental Mouse Models and Confers the Protective Effect of Cruciferous Vegetables in Mice and Humans. *J Am Soc Nephrol* **31**, 102-116 (2020).
5. 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).
6. Ye, L.*, et al.* Quantitative determination of dithiocarbamates in human plasma, serum, erythrocytes and urine: pharmacokinetics of broccoli sprout isothiocyanates in humans. *Clin Chim Acta* **316**, 43-53 (2002).
7. Garte, S.*, et al.* Metabolic gene polymorphism frequencies in control populations. *Cancer Epidemiol Biomarkers Prev* **10**, 1239-1248 (2001).