Phase II trial of effects of the Nutritional Supplement Sulforaphane on Doxorubicin-Associated Cardiac Dysfunction

Specific Aim

Determine whether nutritional supplement sulforaphane (SFN) is safe to administer to breast cancer patients undergoing doxorubicin (DOX) chemotherapy. We have identified biomarkers for presymptomatic detection of DOX cardiotoxicity in breast cancer patients and reported that SFN alleviates DOX-induced cardiac toxicity while maintaining its anti-tumor activity in an animal model of breast cancer. SFN is in various stages of preclinical and clinical trials against different types of cancer but its safety and effects on identified markers have never been tested in patients treated with DOX. Sulforaphane is a generally recognized as a safe (GRAS) compound and this compound is currently in 61 different clinical trials including, Cystic Fibrosis, COPD, Melanoma, Breast and prostate cancer etc. (https://clinicaltrials.gov/ct2/results?term=sulforaphane&pg=1). Avmacol, (Nutramax Laboratories Consumer Care, Inc. Edgewood, MD 21040) an over-the-counter dietary supplement containing broccoli seed and sprout extracts that is rich with sulforaphane, will be used in this study. This is an FDA regulated trial using an investigational drug under IND 141682. Dose considerations were made on the basis of the ongoing clinical trial "Effects of Avmacol® in the Oral Mucosa of Patients Following Curative Treatment for Tobacco-related Head and Neck Cancer" (https://clinicaltrials.gov/ct2/show/NCT03268993). Furthermore, Avmacol has never been tested as an adjuvant to try and protect the heart from DOXs harmful effects. Therefore, we are proposing to do an early-clinical trial to assess SFN safety in DOX-treated patients and possibly prevent DOX-cardiotoxicity in breast cancer patients.

Our **hypothesis** is that SFN protects the heart from DOX-mediated cardiac injury without affecting its antitumor efficacy. Here, we will test SFN in combination with DOX-based chemotherapy for breast cancer; we will assess its safety, cardio-protective properties, and anti-cancer efficacy. We will also examine the effect of SFN on the expression of several biomarkers that indicate when a patient cannot tolerate DOX.[1] To test our hypothesis, we propose the following Specific Aims.

Aim 1: Demonstrate that co-administration of SFN protects the heart and is not associated with toxicity or reduced tumor response in breast cancer patients undergoing DOX chemotherapy.

We propose a pilot clinical trial to assess the safety of SFN as a co-treatment with DOX chemotherapy. Strategy: Up to seventy breast cancer patients prescribed DOX chemotherapy will be consented then randomly assigned to receive either SFN or placebo during DOX chemotherapy. This sample size includes an additional 10 participants to allow for potential attrition. There will be a 50-50 chance of receiving SFN. The patients will be recruited from the Breast Cancer Clinic (Dr. Awasthi) in the Southwest Cancer Center at TTUHSC/UMC Lubbock. Cardiac function with echocardiography will be assessed and compared between arms for evidence of substantive decrease in DOX cardiotoxicity with SFN compared to placebo. Tumor size will also be compared (based on RECIST criteria) between treatment arms. We will monitor treatment-emergent symptoms, hematological parameters of cancer therapy toxicity, and renal and hepatic function. Blood samples will be collected to assay markers of cardiotoxicity, including B-type natriuretic peptide and troponin.

Aim 2: Determine if SFN alters the levels of known biomarkers of DOX cardiotoxicity and affects the expression of SIRT1- and Nrf2-target genes.

We will measure changes in transcript and protein biomarkers of pre-symptomatic DOX-cardiotoxicity in response to SFN, and will determine if SFN promotes SIRT1- and Nrf2-dependent gene expression.

Because Nrf2 upregulates certain detoxifying enzymes, we will measure the plasma levels of cardiotoxic metabolites, doxorubicinol (DOXol). Strategy: Blood will be collected before the start of DOX chemotherapy and after each treatment cycle to isolate peripheral blood mononuclear cells (PBMCs) and prepare plasma. In lieu of tissue biopsies, PBMCs will be used to examine SIRT1 and Nrf2 activity and target gene expression. Multiplex arrays will be used to measure plasma cytokine levels, and plasma DOX metabolites will be assessed with ultrahigh performance liquid chromatography-tandem mass spectrometry. These experiments will provide initial evidence to indicate that SFN activates SIRT1 and Nrf2 pathways in non-cancer tissue of breast cancer patients.

Impact: This will be the first clinical study to assess the safety of SFN as a co-treatment with DOX to mitigate cardiotoxicity in breast cancer patients. Positive findings will be used to justify a larger randomized controlled trial. Subjects will be randomized at a 1:1 ratio.

Background

DOX toxicity and its impact on human health. Cardiotoxic side-effects are among the main complications of doxorubicin chemotherapy and this limits its use in high-dose cancer treatment, and strongly impacts the quality of life and survival of cancer patients.[2-12] The occurrence of cardiac events in cancer patients associated with DOX-therapy is estimated between 10% and 25%.[13,14] The prevalence of left ventricular contractile dysfunction in patients with a cumulative DOX dose of approximately 430–600 mg/m^2 is about 50–60%, in which a significant incidence of cardiomyopathic episodes is observed.[13,14] DOX-induced cardiotoxicity can develop immediately, within months, or even years after completion of therapy.[14] Dexrazoxane is the only USFDA approved agent for reducing DOX-cardiomyopathy in women with metastatic breast cancer, but it has its own side effects.[15,16] Clearly, devising a strategy to prevent DOX-induced cardiotoxicity would have tremendous benefit in terms of limiting medical complications and improving lives of cancer patients.

DOX cardiotoxicity and anti-cancer activity. The mechanism of anti-cancer action of DOX seems mostly distinct from the mechanisms responsible for its cardiotoxicity. DOX anti-cancer activity is based on producing DNA damage and inhibiting cell replication.[17-22] These effects are almost nonexistent in non-replicating cardiomyocytes. Instead, the primary cause of cardiotoxicity is DOX-induced oxidative stress, resulting in part from its inhibition of nuclear factor (erythroid-derived 2)-like 2 (**Nrf2**) expression (also supported by our new findings).[23] DOX exposure causes initial mitochondrial injury, which compromises cellular bioenergetic capacity, and initiates a delayed progression of cardiac damage.[24,25] Since the mechanisms of DOX-mediated cardiotoxicity and anti-cancer activity are different, it is likely that cardiac and cancer cells employ different protective mechanisms. Therefore, elucidations of such mechanisms will not only help in devising novel strategies to protect the heart from the harmful effects of DOX, but also help to combat cancer more effectively.

Sulforaphane (SFN) is protective to normal cells, but toxic to cancer cells. SFN, a safe phytochemical, protects and enhances Nrf2 signaling and consequent cytoprotective gene activation to counter DOX cardiotoxicity in animal models.[23,26-33] SFN also exhibits anti-cancer activities related to enhancing the transcription of tumor suppressor genes, possibly by influencing DNA methylation, activation of caspase-3, induction of cell cycle arrest, reactivation of estrogen receptor alpha (**ERα**), and by inhibition of DNA methyltransferase (**DNMT**) and telomerase reverse transcriptase (**TERT**).[34-42] As well, SFN may possibly influence DOX metabolism in cancer cells to potentiate its anti-tumor activity. While SFN is in various stages of testing in clinical trials of different types of cancer

(https://clinicaltrials.gov/ct2/results?term=sulforaphane&pg=1), [26,42,43] it has not yet been exploited as an adjuvant with doxorubicin in breast cancer treatment regimens.

Overall Rationale

Metastasis and DOX-induced cardiotoxicity are the most common causes of mortality for DOX-treated cancer patients.[44] SFN is a nontoxic compound isolated from cruciferous vegetables that exhibits anticancer, antidiabetic, and antimicrobial activity in many animal models of disease.[26,42,43,45] Our preliminary data suggest that SFN not only protects the heart from DOX toxicity but enhances the effectiveness of DOX against breast tumors *in vivo.*

SFN is in various stages of preclinical and clinical trials against different types of cancer, including prostate cancer.[46] However, its safety has never been tested in patients treated with DOX, and it has never been tested for a cardioprotective effect in combination with DOX. Therefore, we propose a pilot clinical trial to assess the safety and efficacy of SFN in DOX-treated breast cancer patients. In this trial, we will assess cardiac function as an indicator of whether SFN prevents DOX-induced cardiotoxicity.

Preliminary Studies

SFN prevents DOX-induced cardiomyopathy in tumor-bearing rats (Accepted manuscript in PLOS one). Because DOX is used to treat breast cancer in humans, we examined the cardioprotective action of SFN during DOX chemotherapy in a rat model of breast cancer. MAT B III breast cancer cells were injected into a mammary gland of female Sprague Dawley rats (6-8 weeks old), and the animals were treated when tumors reached 5-6 mm^3 in diameter.[47] SFN (4 mg/kg in 0.1 ml saline) or vehicle was administered orally 5 days/week for 5 weeks. After 1 week of SFN (or vehicle) treatment, the rats were injected intraperitoneally with DOX (5 mg/kg) or vehicle once per week for the remaining 3 weeks (total of 20 mg/kg, similar to a dose of 120 mg/m^2 for humans). Two days after completing treatment, cardiac function was assayed, and tissues were collected for further analysis. DOX treatment dramatically reduced cardiac contractility, as measured by fractional shortening (FS%), left ventricular ejection fraction (LVEF%), cardiac output (CO), and stroke volume (SV). The cardiotoxic effects of DOX were significantly attenuated with SFN co-treatment (**Fig. 1**).

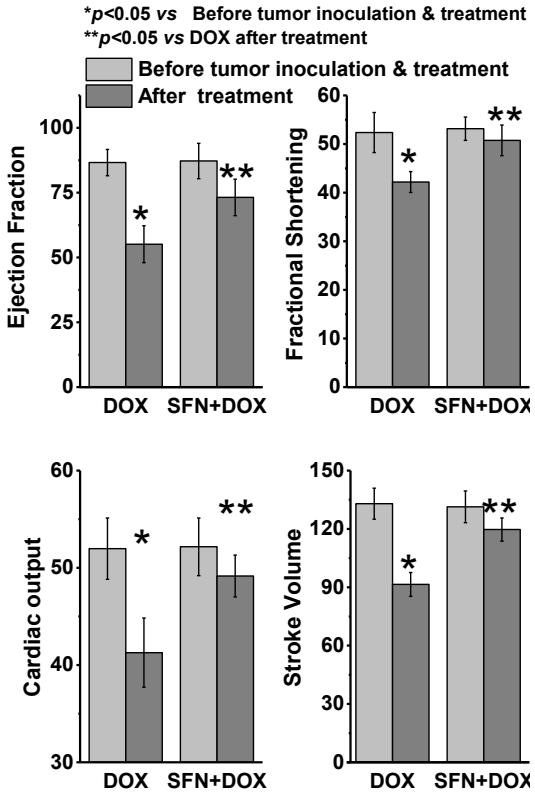

Figure 1. SFN+DOX co-treatment of tumor-bearing rats preserves cardiac function. MAT B III breast cancer cells were injected into a mammary gland of adult rats (n = 8). SFN (4 mg/kg in 0.1 ml saline) or vehicle was administered orally 5 days/week for 5 weeks. After 1 week, rats were injected i.p. with DOX (5 mg/kg or vehicle once per week for the remaining 3 weeks. Cardiac function was assessed with high-resolution echocardiography (Vevo 700) before tumor inoculation and treatment and after the final treatment.

SFN enhances the ability of DOX to regress breast tumors. To determine if SFN affected the anticancer activity of DOX, we treated tumor-bearing rats with vehicle, SFN, DOX, or SFN+DOX (as just described) and monitored tumor growth. SFN alone had no significant effects on tumor growth at the doses intended in the study (albeit it does have mild antitumor activity at higher doses), and DOX alone reduced tumor growth (**Fig. 2A**). However, SFN+DOX eradicated the tumors from all rats by day 35 after tumor implantation (**Fig. 2A**). Thus, SFN co-treatment does not impair the ability of DOX to kill cancer cells, and SFN can be used safely with DOX to attenuate cardiotoxicity. We also found that the DOX dose can be lowered by 50% when combined with SFN to eradicate tumors (**Fig. 2B**). Thus, SFN+DOX co-treatment reduces DOX cardiotoxicity and decreases tumor volume in rats.

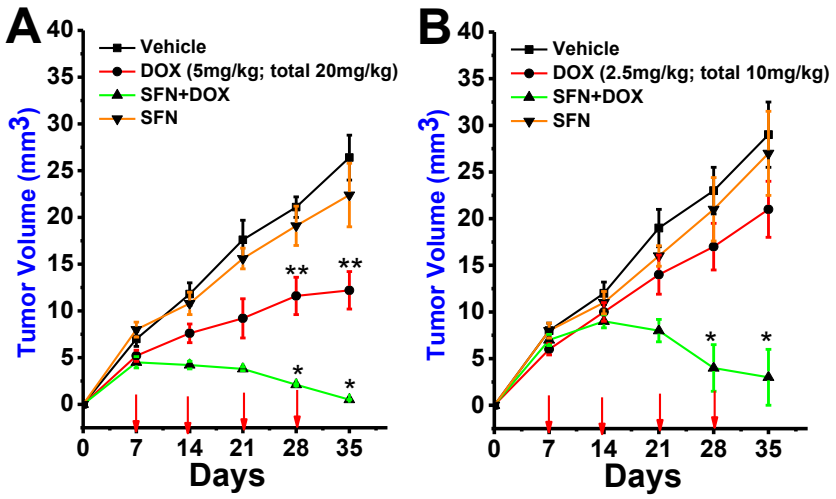

Figure 2. SFN co-treatment lowers the effective DOX dosage in a rat breast cancer model. Effect of metronomic SFN (4 mg/kg, 5 days/week), DOX (**A**, 5 mg/kg weekly or **B**, 2.5 mg/kg weekly, total 10 or 20 mg/kg), or SFN+DOX on MAT B III-induced mammary tumors. Data represent the mean tumor volume ± SD. Red arrows indicate DOX injections. (*p = 0.002 [SFN+DOX vs. DOX], **p = 0.003 [Vehicle vs. DOX]; n = 8).

SFN improves mitochondrial function during DOX treatment. In cardiac cells, DOX is converted to a more reactive semiquinone by mitochondrial complex I of the electron transport chain (ETC), increasing oxidative stress.[48-51] We reasoned that these effects on the ETC decrease the level of ATP in cardiomyocytes. However, SFN is known to increase respiration linked to ATP synthesis.[52-55] To determine if SFN stabilizes ATP levels in the cardiomyocytes of DOX-treated rats, we examined ATP levels in treated and control animals. We found that SFN maintains ATP levels during DOX treatment (**Fig. 3A**).

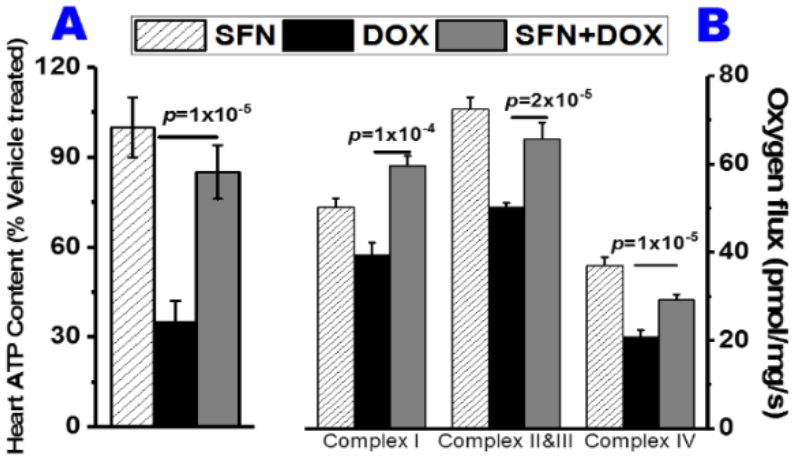

Figure 3. SFN increases ATP content in the rat heart during DOX treatment. A) ATP content was evaluated in fresh heart biopsies. **B)** SFN improves ETC function during DOX treatment. According to oxygen flux measures, co-treatment of rats with SFN+DOX improved complex I, II+III, and IV respiration compared to rats treated only with DOX. Each bar represents the mean ± SD (n = 8); statistical significance determined with a t-test.

Pharmacological activation of Nrf2-target gene expression by SFN should remove excess ROS produced during DOX therapy by elevating phase 2 detoxification and antioxidant enzyme activities and preserving ETC complex function. To evaluate this, ETC activity was measured in left ventricular biopsies of SFN- and/or DOX-treated rats with high-resolution respirometry using the OROBOROS Oxygraph-2k (Oroboros Instruments).[56] SFN clearly protected the ETC from damage by DOX (**Fig. 3B**), as indicated by an increase in oxygen flux. We confirmed that these protective effects were mediated by Nrf2-dependent signaling by comparing the enzymatic activities of SOD and catalase, two Nrf2 target gene products (data not shown).

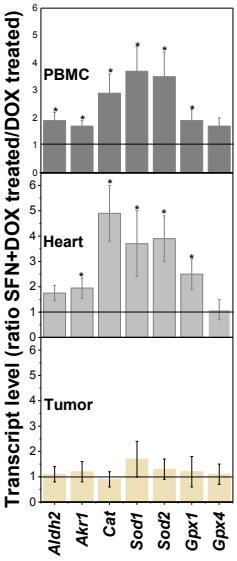

Figure 4. Differential expression of antioxidant and antielectrophilic genes in SFN+DOX and DOX-treated tumor-bearing rats. Transcript levels were measured with qRT-PCR on 6 animals per group and were normalized to *Rsp3* for each animal. The bars represent ratios (± SD of the ratio) of mean transcript levels of statistically significant genes. $p < .05$; values were calculated with two-factor ANOVA on the basis of two independent experiments.

SFN upregulates key Nrf2-target antioxidant genes in the PBMCs of DOX-treated tumor-bearing rats. SFN preserves nuclear Nrf2 activity in DOX-treated rat cardiomyocytes but not in DOX-treated breast tumor tissue (data not shown), and this promotes the upregulation of antioxidative and antielectrophile defenses in heart cells. Notably, we demonstrated that the gene-expression profiles of cardiomyocytes and peripheral blood mononuclear cells (PBMCs) are very similar but differ from the expression profile of breast tumors,[22] and this supports our approach to use PBMCs as a surrogate tissue for assessing biomarkers of DOX cardiotoxicity (**Fig. 4**).

Specifically, we compared the transcript levels of key Nrf2-target mitochondrial antioxidant/antielectrophile genes between tumor cells, cardiomyocytes, and PBMCs of DOX- and SFN+DOX-treated tumor-bearing rats. In the heart and PBMCs, there were significant increases in the expression of key antioxidant and antielectrophilic genes (**Fig. 4**). However, transcript levels of these Nrf2-dependent enzymes were unchanged in the SFN-treated tumor tissue. These data suggest that protective Nrf2 signaling is activated in the cardiomyocytes and PBMCs of DOX-treated animals, not in tumor cells.

We conclude that SFN does not induce protective mechanisms in tumor cells, and we predict that it can be used safely as an adjuvant with DOX.

Above animal studies, by our University of Arkansas for Medical Sciences (UAMS) group has provided strong evidence that DOX-toxicity correlates with impairment of antioxidant defenses caused by a defect in functions of Nrf2.[23,57] Nrf2 is a regulatory protein that coordinates the body's defense mechanisms against oxidative stress and its resultant damage. SFN, an Nrf2-related activator of cytoprotective genes, provides protection in several disease states[26,30,31,33] and is in various stages of clinical trials.[26,43,58-64] SFN is a metabolic product of glucoraphanin, a glucosinolate found in broccoli and cauliflower, is commercially available as a dietary supplement, and has an excellent safety profile. We recently reported that elevated Nrf2 activity drives cellular defenses to provide protection against DOX-induced cardiomyopathy.[57] In addition, our pilot studies in cardiac cells reveal that SFN modifies PGAM5, initiating nuclear translocation of Nrf2 to activate protective gene expression and confer resistance to DOX. Also, we discovered that SFN therapy protected rats from DOX-induced cardiomyopathy. In an additional study in rats, SFN enhanced the chemotherapeutic effects of DOX on implanted breast cancer cells.

These preliminary studies are exciting and could have immense translational impact in terms of use of SFN to lessen DOX-toxicity; however, it has never been tested in patients receiving DOX, and it has not yet been exploited as an adjuvant in cancer treatment regimens. Therefore, we propose to use SFN in breast cancer individuals undergoing DOX-therapy to see if it will alleviate DOX-associated oxidative damage and improve cardiac function. Our goal is to aid in the development of a safe strategy to reduce cardiac side effects of DOX.

Study Design and Procedures

Study Design. We intend to include up to 70 DOX-naïve women diagnosed with breast cancer undergoing neoadjuvant chemotherapy with no prior cardiac disease and who will receive DOX without Her-2 receptor antagonists (to eliminate possibility of secondary side effects) as part of their clinical care. These potential subjects will be recruited in a randomized, controlled, double blinded pilot study comparing SFN to placebo. The study will be conducted at the Texas Tech University Health Sciences Center, and University Medical Center. The trial will be approved by our local IRB and registered at clinicaltrials.gov. UMC cancer center pharmacist Ajoke A. Tijani, RPh. (Tijani@umchealthsystem.com) will receive placebo and test compound from supplier and will dispense them to test subjects.

Randomization will be achieved with assignment of a subject ID to the study subject. The list of subject IDs will be subsequently assigned either study drug or placebo using a randomizer software such as (www.randomizer.org). The pharmacist dispensing the medication will be aware of the study IDs in relation to the patient identity, in order to be able to dispense the test drug or the placebo per their assigned status as test or control subjects.

Study Drug and Placebo. Processed SFN-rich extract will be purchased in form of caplets from Nutramax Laboratories, Inc. 2208 Lakeside Blvd Edgewood, MD 21040. Caplets containing SFN-rich broccoli sprout extracts or microcrystalline cellulose (placebo) also from Nutramax Labs will be dispensed to participants in sealed bottles with instructions to keep them in a household freezer. Size of the caplet will be about the size of a 1000 mg Vit C pill (about 2 cm in length). The participants will be dosed, based on weight, in a double-blind fashion with identical appearing placebo or SFN caplets in a daily dose for 12 weeks of: two caplets for individuals <100 lb., four caplets for individuals 100–200 lb. and eight caplets for individuals >200 lb. Avmacol or placebo will be prescribed by Dr. Awasthi and will be dispensed by local pharmacy or study coordinators at TTUHSC/UMC Lubbock. We will be doing pill counts to make sure that volunteers have used as directed. We will measure the Sulforaphane level in plasma by well-established method. [26]

The drug and the placebo will be stored at the South West Cancer Center pharmacy based on manufacturer based guidelines and dispensed to each participant at their baseline visit then at DOX infusion visits 1, 2, and 3. Patients will be expected to maintain at least an 80% adherence to the medication regimen, in the absence of prohibitive toxicity. Adherence will be monitored through therapeutic drug level (Plasma sulforaphane levels of 120 ng/ml) monitoring as mentioned above and pill counts. For subjects who do not meet 80% compliance, they will be instructed on the importance of taking the pills as directed, but if their next visit demonstrates less than 80% compliance, they will be withdrawn from the study. As long as participants complete their 4th cycle of DOX chemotherapy treatment we will still use their study data during analysis. If side effects are noted, patient will be asked to notify the study team of the same and will be evaluated within a suitable time frame based on severity of side effects.

SFN is a safe natural isolate. Above doses (30mg/caplet) are considered adequate to maintain intended therapeutic drug levels, while maintaining a simple study design and without significant concerns for drug toxicity It has been used in several clinical trials from doses ranging from 2-200 µmol/day for 2-28 weeks in 25-80 years old subjects without significant side-effects or toxicity (https://clinicaltrials.gov/ct2/results?term=sulforaphane&Search=Search), yet never in combination with DOX.

Statistical Considerations

Data for the primary endpoint (DOX cardiotoxicity) and other binary outcomes will be summarized by treatment arm as number and proportion per arm. The change in DOX-cardiotoxicity rate with SFN compared to placebo will be assessed with a 1-sided *Z*-test (see *Sample-size justification*). Tumor size, plasma cardiac biomarkers, cardiac ejection fraction, DOX metabolite levels will be summarized by treatment arm and time point as means and standard deviations and graphed as box plots and profile plots. We will adhere to ANOVA distributional assumptions so that appropriate data transformations can be applied. Data for each continuous variable will be analyzed for differences in group means at each time point with ANOVA or Mann-Whitney-Wilcoxon non-parametric tests at each time point. These tests will employ a more-stringent alpha = 0.02 significance level to adjust for the multiple comparisons without overinflating type II error. For subjects withdrawn from the study or do not complete the study for any reason, as long as they completed their 4th cycle of DOX chemotherapy treatment we will still use their study data during analysis.

Sample-size justification. The number of subjects (35 per treatment arm; 70 total; we expect some attrition, which this sample size addresses) is based on the primary endpoint of DOX-induced cardiac dysfunction, the rate of which was recently observed at our previous institution (UAMS) to be ~25%[1]. We expect that SFN will decrease this to 5%. The cardiotoxicity rates for each arm will be compared via 1-sided pooled-variance *Z*-test at an alpha = 0.10. The statistical power of this test, conducted as described, needs to exceed 80% in order for our study to be generally recognized as having adequate statistical power. Our sample size provides the 1-sided pooled-variance *Z*-test with 83.2% power at 10% alpha to detect the expected 20-point decrease in DOX-toxicity rates from 25% in the placebo arm to 5% in the SFN arm.

The formal consent of each subject, using the IRB-approved consent form, will be obtained before that subject is submitted to any study procedure. The study schedule is detailed in Table 1.

Table 1: Study Schedule

Procedures	**Screening**	**Baseline**	**First DOX infusion**	**Second DOX infusion**	**Third Dox infusion**	**Fourth Dox Infusion**	**1 week S/P 4th Dox infusion**	**1 year follow-up**
		Day 0 (within 3 weeks +/- 3 days from screening)	1st week +/- 3 days	Week 3rd-4th +/- 3 days	Week 5th-6th +/- 3 days	Week 7th-8th +/- 3 days	Week 8th-9th +/- 3 days	Week 60-62 +/- 3 weeks.
ICF	X							
HX review	X							
EKG	X							X
ECHO	X						X	X
PET CT	X						X	
Urine pregnancy test	X							
I&E review		X						
Randomization to SFN or Placebo		X						
SF 36 questionnaire		X					X	
Blood work (CBCd, CMP, Research) If still on DOX, draw prior to DOX Chemotherapy infusions		X (prior to first dose of study medication)	X	X	X	X	X	X
Weight		X	X	X	X	X	X	X
Dispense Study medication. (# of tablets based on weight criteria see below)		X	X	X	X			
Pill count			X	X	X	X		
Assess Adverse events			X	X	X	X	X	X

DOX Chemotherapy goal is every 2 week infusions Week 1, Week 3, Week 5, and Week 7. This is Dose- Dense Chemotherapy only (for a uniform patient population on uniform chemotherapy dosing).
WT = <100 pounds 2 tablets per day
WT 100 to 200 pounds 4 tablets per day
WT greater than 200 pounds 8 tablets per day

Goal for medication compliance is =/over 80%

The study drug (SFN or placebo) will be administered for a total of 12 weeks. Patients will be monitored every 2 weeks at their DOX chemotherapy infusion visits for toxicity checks for the initial 8 weeks and "as needed" thereafter. Please refer to "safety monitoring" topic for further details.

Study subjects will be instructed to avoid taking all over the counter supplements during the 12 weeks of the study when taking the trial drug/placebo.

Questionnaires

The subject will complete a general health questionnaire (SF 36) at the baseline visit and a week after completing the 4th infusion. Non-English speakers will be provided a translator (either in person or on phone/video) to aid with filling out the questionnaire.

Blood samples. Approximately 20 ml of non-fasting blood will be drawn from the participants by someone trained in phlebotomy: about 10 ml of this sample will be used for routine laboratory monitoring (complete blood count with differential and comprehensive metabolic panel). Remaining sample will be used for doxorubicin associated cardiotoxicity assessment as detailed below. Blood will be collected at:

- Before starting SFN (or placebo) at baseline
- Before the first DOX chemotherapy infusion
- Before the second DOX chemotherapy infusion
- Before the third DOX chemotherapy infusion
- Before the fourth DOX chemotherapy infusion
- About one week after the fourth DOX chemotherapy infusion
- About one year after completion of DOX chemotherapy

Peripheral blood cells (PBCs) are isolated from EDTA anti-coagulated blood using standard Ficoll-Paque Plus gradient centrifugation (density 1.073 g/mL) according to the instructions of the manufacturer (GE Healthcare, USA). Total RNA will be extracted from PBC using RNeasy columns (Qiagen; Valencia, CA) and samples with RNA integrity number (RIN) score>7 will be used for gene expression analysis by qPCR. Differentially expressed genes (**DEG**) of patients who developed DOX-associated cardiotoxicity after the completion of chemotherapy were compared with DEG of patients who did not. We have identified 67 unique up or down regulated genes. The most enriched "biological functions" of this dataset were cell-to-cell signaling, cellular movement, free radical scavenging, inflammatory diseases, skeletal and muscular disorders, immunological diseases and cellular development. We will perform qRT-PCR to determine the effect of SFN on identified genes in patients with abnormal LVEF in comparison with the patients with normal LVEF.

2D ECHO - We will evaluate cardiac function in the oncology clinic at UMC Lubbock with 2D echo. 2D echo is undertaken when cardiac structural details are desired alongside an estimation of cardiac ejection fraction. 2D echo will be done with either a GE Logic E9 or a Philips Epic machine by experienced technicians. One blinded cardiologist, certified in reading ECHO, will read the report. The same machine and protocol will be used for all patients.

ECHO will be done per standard of care:

- before starting SFN (or placebo) at baseline
- one week after the forth DOX chemotherapy infusion

- one year after completion of DOX chemotherapy

FDG – PET CT

We will evaluate tumor response objectively by baseline and post treatment (one week +/- 3 days from 4th DOX infusion) completion FDG – PET CT. Testing will be done in house by a radiologist with expertise in FDG-PET. He will be blinded to the treatment regimen as well.

Evaluation of tumor response will be based on standard PERCIST (version 1.0) criteria.

https://pubs.rsna.org/doi/10.1148/radiol.2016142043

The FDG – PET CT, questionnaires, specific blood draws, and the nutritional supplements or placebos are study related. All other tests and activities are part of routine breast cancer treatment care.

Sulforaphane and Placebo Information

SFN in the precursor glucosinolate form is widely available as a nutritional supplement. However, effective conversion to the isothiocyanate form depends on the intestinal flora and may vary among individuals. Therefore, we will administer Avmacol®, an innovative supplement that contains glucoraphanin and active myrosinase enzyme to maximize and stabilize the amount of SFN provided to each patient. Data on humans provided by the manufacturer has shown that Avmacol® creates an average of 44± 4 micromoles of SFN per recommended serving (2 caplets). [65-71] Avmacol® or microcrystalline cellulose (placebo) will be purchased in form of caplets from Nutramax Laboratories, Inc. (Edgewood, MD). Placebo and test compound will be dispensed to participants in sealed bottles with instructions to keep them in a household freezer. The participants will be dosed in a double-blind fashion with identical appearing placebo or SFN caplets in a dose of two caplets for individuals with weight of <100 lb, four caplets for individuals with a weight of 100–200 lb, and eight caplets for individuals weighing >200 lb daily for 12 weeks. UMC Cancer Center (Lubbock) pharmacist Ajoke A. Tijani, RPh. will receive and store placebo and test compound from the supplier.

Study Population

We intend to include seventy DOX-naïve breast cancer patients undergoing neoadjuvant chemotherapy with no prior cardiac disease and who will receive doxorubicin without Her-2 receptor antagonists as part of their clinical care will be recruited in a randomized controlled, double blinded pilot study comparing SFN to placebo. Study participants will be randomized 1:1 to receive placebo or test compound.

Inclusion Criteria

- Age 18 to 89 years
- No prior diagnosis of coronary artery, carotid artery or peripheral artery disease
- Not pregnant or breastfeeding (urine pregnancy test will be done if female of childbearing potential)
- Breast cancer requiring treatment with DOX-containing regimen above
- Women in child bearing age group (18-50 years) will agree to use birth control for duration of study
- Study subjects must be willing and able to swallow caplets, up to 8 daily.

Exclusion Criteria

- Pregnant (by urine pregnancy test)
- Baseline ejection fraction of less than 50%, evidence of left ventricular hypertrophy or baseline EKG reported as abnormal per cardiologist.
- Inability to provide informed consent.
- Prior history of chest radiation therapy
- Diabetes or Hypertension or prior Myocardial infarction
- Trastuzumab patients
- Taking other supplements
- Inability to follow up for safety monitoring
- Prisoners
- Previous or current use of cocaine or any illicit drug
- Unable or unwilling to provide blood samples
- Taking medications known to have cardiac effects, such as but not limited to, beta blockers, anti-arrhythmic agents, non dihydropyridine calcium channel blockers, ace inhibitors, NSAIDS, diuretic agents.
- Unable to follow the protocol
- Inability to receive anthracycline due to any reason (underlying baseline cardiac dysfunction due to other reasons, with an EF under 50%)
- Patients already taking SFN OTC

Potential Benefits

While no direct benefit to the study participants is intended, the primary effects of sulforaphane in conjunction with doxorubicin may provide additional benefits to that particular patient group. However, the magnitude of such benefit, if any, is hypothetical and hence the need for above testing for further confirmation.

This study will shed light on the role of anti-oxidant therapy (Sulforaphane) in improving cardiac function in the patients undergoing DOX-therapy. It may provide a safe, readily available resource to potentially reduce the decline in cardiac function with DOX chemotherapy.

Potential Risks

- Sulforaphane: This product has a very safe effect profile and is available over the counter as a nutritional supplement. Previous clinical trials do not report any serious side effect this supplement except some minor incidents of vomiting, abdominal pain, constipation, increased appetite etc. (https://clinicaltrials.gov/ct2/show/results/NCT01474993?sect=X430156#othr). Since it has not been tested in breast cancer patients before, patients will be monitored for any previously unreported side effects. The complete blood count (CBC), and CMP tests will be used as a broad screening test to determine an individual's general health status.

- Microcrystalline cellulose (placebo): This product is chemically an inert substance, if taken in large quantities it can cause diarrhea in some individuals.
- Blood Draw: Venipuncture can cause some pain, discomfort, redness, infection, bleeding, bruising and swelling at the draw site, lightheadedness, or fainting

As with most studies, there is also a potential risk of breach of confidentiality.

Safety Monitoring

A physician investigator will monitor study subjects prior to each treatment dose (about every 2 weeks) for assessment of tolerance, possible side effects and review of laboratory data (complete blood count with differential and comprehensive metabolic panel). Patients will also be provided with a hotline number where a physician will be accessible 24 hours a day and 7 days a week for reporting possible serious adverse effects. Such calls will then be triaged by the physician (symptomatic treatment versus ER visit) based on type and severity of symptoms reported. Such events will be noted in the patient's file and will be reported in the trial results.

It will be at the discretion of the physician investigator to delay or discontinue treatment depending on the severity of the noted adverse effects (delayed for up to grade 3 CTCAE and discontinued for grade 4 CTCAE events).

All adverse events including side effects attributed to treatment, treatment delays or discontinuation will be duly recorded in the study records for further reference.

Adverse events will be reported to the IRB as described in the TTUHSC Human Research Protection Manual.

Data Handling and Recordkeeping

The Principal Investigator will carefully monitor study procedures to protect the safety of research subjects, the quality of the data and the integrity of the study. All study subject material will be assigned a unique identifying code or number (study ID). The key to the code will be kept in a locked file in the Principal Investigator's office, if hardcopy, or on a password-protected TTUHSC server (iRIS), secured behind the TTUHSC firewall, both located behind locked doors in a restricted access area of the TTUHSC campus. Only authorized individuals will have access to the code and information that identifies the subject in this study. Data will be kept for 3 years as per TTUHSC IRB regulations.

Basic health screening data will be collected using standardized paper forms that will be identified with the participant's study ID. Paper data will be entered into the computer independently by research staff trained to perform data entry. The computer data will be stored on a secure drive behind the TTUHSC firewall and downloaded by the Data Manager, who will then transfer the data to SPSS and check for missing data. The computer(s) used to collect data will be kept in a locked office or locked cabinet when not in use.

Blood samples, identified only with subject ID, will be obtained and analyzed in the investigator's lab. Results of the assays along with week, date, and time of sample collection will be entered into the study dataset. The Data manager (Sharda Singh) will employ a verification program to determine and correct any discrepancies based on source data. The study monitor will also help with data verification. Echocardiograms will be identified using participant study ID only and will be stored on the archiving system of the cardiac noninvasive lab at TTUHSC. All analysis will be performed in the lab and the

results will be entered in a paper form with the patient ID.

Electronic and hardcopy study data will be maintained as explained above until manuscript publication, after which electronic files will be erased from the TTUHSC server and hard copy documentation will be shredded according to TTUHSC Policy. Blood samples not completely consumed by analyses will be mixed with sodium hypochlorite solution and disposed of down a sink drain according to TTUHSC policy.

Ethical Considerations

This study will be conducted in accordance with all applicable government regulations and Texas Tech University Health Sciences Center research policies and procedures. This protocol and any amendments will be submitted and approved by the TTUHSC Institutional Review Board (IRB) to conduct the study.

The informed consent of each subject, using the IRB-approved consent form, will be obtained before that subject is submitted to any study procedure. The consent form will be translated into Spanish to accommodate Spanish reading only participants; and a Spanish translator will be used when necessary to facilitate communications. All subjects for this study will be provided a consent form describing this study and providing sufficient information in language suitable for subjects to make an informed decision about their participation in this study. The person obtaining consent will thoroughly explain each element of the document and outline the risks and benefits, alternate treatment(s), and requirements of the study. The consent process will take place in a quiet and private location, and subjects may take as much time as needed to make a decision about their participation. Participation privacy will be maintained and questions regarding participation will be answered. No coercion or undue influence will be used in the consent process. A copy of the signed consent will be given to the participant, scanned into his or her medical record and the iRIS system; and the informed consent process will be documented in each subject's medical record.

Monitoring Plan:

To ensure compliance of the study protocol, GCP guidelines, and TTUHSC Human Research Protection Program research policies and procedures during the conduct of the study, and quality data a monitor in the Clinical Research Institute will conduct the monitoring of the study. The first monitoring visit will be conducted within two weeks after the first subject has been enrolled into the study. The succeeding monitoring visits will be scheduled periodically, but no less than every 2 months when there is an active study participant, at a mutually agreed timeframe by the PI and study monitor. All data collected will be 100% source document verified. The study monitor may inspect and audit all study documents, i.e. data collection forms, drug accountability, and medical records, within the applicable confidentiality regulations.

Dissemination of Data

Results of this study may be used for presentations, posters, or publications. The publications will not contain any identifiable information that could be linked to a participant.

Funding: This IRB protocol approval is needed to submit a grant application to NIH, DoD and intramural funding. Approved study will start upon receipt of the funds. The use of Clinical Research Institute resources will be acknowledged in any publications.

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