**Supplementary Materials and Methods**

**Preparation of BSE beverages**

The clinical study administered beverages prepared from rehydrated, lyophilized, sulforaphane-rich broccoli sprout powder as previously described ([1](#_ENREF_1)). Briefly, powder was produced by the Cullman Chemoprotection Center at the Johns Hopkins University School of Medicine for clinical studies as an Investigational New Drug (Kensler; 119646). Broccoli (*Brassica oleracea* var. *italic*) sprouts were grown from well-vetted BroccoSproutsTM seeds (cultivar DM1999B) with technology licensed from Johns Hopkins University. Seeds were grown in a commercial sprouting facility under controlled conditions. An aqueous extract containing about 5 mmol/L glucoraphanin was prepared from 3-day old sprouts in a steam-jacketed kettle at Oregon Freeze Dry, a GMP food processing facility. To prepare glucoraphanin-rich powder, the aqueous extract was filtered and lyophilized. Total glucoraphanin content, as determined by high-performance liquid chromatography (HPLC;([2](#_ENREF_2))), was 329 μmol/g powder. To prepare sulforaphane-rich powder, the aqueous extract was filtered, cooled, and hydrolyzed with myrosinase derived from daikon sprouts (*Raphanus sativus*), then lyophilized. Total isothiocyanate and sulforaphane content were quantified by cyclocondensation analysis ([3](#_ENREF_3)) and by direct HPLC ([4](#_ENREF_4)), respectively. Sulforaphane content was 202 μmol/g powder and represented 91% of the total isothiocyanate content.

The bulk powders were tested for microbial contaminants (IEH-JL Analytical Services and Eurofins Strasburger and Siegel), heavy metals (Elemental Analysis, Inc.), and benzene (TestAmerica). Bulk powder was stored in sealed bags in a locked, dedicated -20°C freezer until reconstitution into the study beverages. To prepare thirty 600 mol doses of glucoraphanin-rich beverage, 18,000 mol of glucoraphanin-rich powder was dissolved in distilled water. Pineapple juice (Dole) and lime juice (Safeway) were added for a final volume ratio of 47:47:6 water:pineapple juice:lime juice. 100-mL individual doses were then transferred into sterile 220-mL commercial bottles. To prepare sixty 150 mol doses of the sulforaphane-rich beverage, 9000 mol of sulforaphane-rich powder was dissolved in distilled water then the same procedure was followed. Individual doses were labeled and stored in a dedicated -20°C freezer in the Investigational Drug Services Pharmacy of the University of Pittsburgh Cancer Institute until dispensed.

**Clinical trial eligibility criteria, participant characteristics, and adverse effects**

This pilot study aimed to provide preliminary data regarding NRF2 pathway modulation in serial buccal mucosa scrapings, in response to short term exposure to three BSE regimens among 10 healthy volunteers. All participants were administered the same sequence of BSE-containing beverages. The primary objective was the feasibility of detecting upregulation of mRNA transcript levels of NRF2-regulated gene, *NQO1*, by qPCR in serial buccal scrapings. Transcript upregulation was defined as 2-fold or greater induction as compared to baseline by quantitative PCR (qPCR), on day 3, 4 or 5 of the BSE regimen. The secondary objective was to estimate the bioavailability of the three BSE regimens, by evaluating sulforaphane metabolites in urine at the conclusion of each regimen.

Eligible participants met the following key inclusion criteria: Healthy male and female adults of any race/ethnicity; aged ≥ 18 years; Karnofsky Performance Scale of ≥ 90%; no current or former diagnosis of invasive cancer; no chronic use of steroids, non-steroidal anti-inflammatory drugs, or CYP3A4 inducers/inhibitors; willing to avoid cruciferous vegetables, multivitamins, and grapefruit for the duration of study interventions. Participants received a $25 gift card upon completion of study interventions.

In March 2014, 12 healthy volunteers consented and 10 were eligible for enrollment onto the pilot study. Baseline characteristics were as follows: 6 females/4 males; 7 Caucasians/3 Asians; 8 never/1 former /1 current smoker; median age 40 (range 27-53). Compliance was excellent in regimens 1 and 2 (oral ingestion of glucoraphanin-rich and sulforaphane-rich beverages, respectively); all participants completed 100% of doses. Compliance with regimen 3 (swish and spit; topical exposure to sulforaphane-rich beverage) was moderate; median time of topical mucosal exposure was 6 of 6 planned minutes (range 1.5 – 7.5 minutes). Compliance with specimen collection was optimal throughout; all required buccal and urine specimens were submitted. Adverse effects were queried daily, and graded according to NCI CTCAE v.4. If Grade 2 or 3 toxicities were encountered, administration of the study beverage was to be discontinued. Adverse effects were queried daily, and graded according to NCI CTCAE v.4. If Grade 2 or 3 toxicities were encountered, administration of the study beverage was to be discontinued. No Grade ≥ 2 toxicities were reported. During regimen 1, one participant reported a self-limited, grade 1 maculopapular and pruritic forearm rash attributed to animal contact. During regimen 3 (swish/spit), 6 participants described grade 1 gum, buccal or pharyngeal irritation attributed to the acidity of the pineapple and lime juice vehicle.

**References**

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