

Blood , Tissue and Toenail Specimens on SELECT

Blood

At randomization, all participants were asked to have a blood specimen collected. The informed consent for the trial had questions about how the specimen could be used: all research, cancer-related research, and research of other health conditions. The answers to these questions are stored and only specimens from those participants who agreed to have their specimen analyzed for the intended purpose will be released. Approximately 84% of participants submitted a usable specimen.

A post-randomization blood draw for all participants was added to trial procedures mid-way through the trial. This additional blood draw was to enable future research that could investigate change over time. There are usable post-randomization bloods on approximately 60% of the randomized participants.

A final specimen collection was requested of all men diagnosed with prostate cancer. This specimen was collected at the participant's final exit visit from their study site.

In addition, all participants at a limited number "representative" sites were asked to submit additional bloods at 6-months, and years 1, 2, 4, 6, 8 and 10 (although the trial stopped short of the 8 and 10-year draw). These 23 sites covered 2,771 participants and constituted the **Adherence Cohort**. These samples were analyzed for plasma levels of selenium and tocopherols and were used for monitoring adherence to the trial supplements.

Blood Collection and Processing

Approximately 20 ml of blood was collected into three 7 ml vacutainer tubes containing EDTA as anticoagulant and protected from light. Bloods were collected at least 3 hours after a meal (i.e., a modified fast), and the date and time of blood collection, as well as time since last meal, was recorded. Modified ice-pack styrofoam shipping boxes were used to keep blood chilled but not frozen during transport, to maintain the integrity of biomarkers of interest (micronutrients, hormones, proteins, and viable lymphocytes).

Blood samples were shipped by overnight delivery to the NCI-Frederick Cancer Research and Development Center for processing and storage. Plasma was divided into up to twenty 0.5 ml aliquots and frozen at -70°C. Buffy coat WBC's were collected and separated into 3-4 aliquots: in two aliquots DMSO was added before freezing at a controlled rate in vapor-phase liquid nitrogen; 1-2 aliquots were frozen at -70°C for later DNA extraction. Two large aliquots (4.5 ml) of RBC's were also stored (-70°C).

Tissue

The Core Pathology Lab for SELECT was responsible for centralized review and confirmation of all positive diagnoses and to coordinate the collection of prostate tissue samples from biopsy, radical prostatectomy or transurethral resection procedures that resulted from the trial. All specimens with a diagnosis of cancer, high grade prostatic intraepithelial neoplasia (HGPIN) or which are suspicious for

cancer diagnosis were reviewed. In addition, a cohort of benign samples was reviewed for quality assurance purposes and to collect control tissue for future ancillary studies.

Tissue from biopsy, prostatectomy and transurethral resection specimens was obtained at study sites using the standard procedures in place at each site. All sites submitted material in neutral buffered formalin fixative for processing and mounting onto glass slides by local pathology laboratories. Some sites also obtained frozen tissue from prostatectomy specimens. After the slides were reviewed by the local pathologists, the diagnostic or representative slides were shipped to the Core Pathology Lab for centralized review. Sites were also encouraged to send additional unstained sections or blocks of the diagnostic tissue to the Core Lab for archiving. At the Core lab each slide was evaluated for the presence of carcinoma, HGPIN, acute and chronic inflammation, atrophy, and hyperplasia. The data was collected on a check sheet and entered into the pathology database and linked with the archived tissue using unique tissue identification numbers. All unstained slides are stored in the environmentally controlled and secure Biorepository Core Facility at the University of Colorado Anschutz Medical Campus. Frozen tissues are stored in vapor phase liquid nitrogen.

Toenail Collection and Processing

Toenail samples were taken at baseline from the great toe and all smaller toes, and stored at room temperature. 89.5% of men have a toenail specimen stored.