D2d Ancillary Study Application

Office Use
Ancillary Study Number: AS14-01 Date Submitted (MM/DD/YYYY): 04/08/2014

Title of Proposal *(81 character limit)*: Biomarkers of cancer in the D2d trial

Principal Investigator: Joel B. Mason, M.D.
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If Principal Investigator is not a member of the D2d study group, please specify:
D2d Co-Investigator: Anastassios Pittas, M.D.
Institutional Affiliation: Tufts University School of Medicine

Other Key Persons (biosketch is not required)
Name: Jerry Dallal, Ph.D. Ancillary Study Role: Biostatitician
Name: Ancillary Study Role:
Name: Ancillary Study Role:
Name: Ancillary Study Role:

D2d Ancillary Study Subcommittee Use Only

Ancillary Studies Subcommittee Action: Please Select Date
Steering Committee Action: Please Select Date
DSMB Action: Please Select Date

Approved Applications

Consent form required? Please Select

D2d Ancillary Study Application

Part 1: Research Plan

1a. Anticipated Timeline and Enrollment

Planned Start Date	01/01/2015
Planned End Date	12/31/2018
Anticipated Enrollment	2,382

1b. Hypothesis and Specific Aims

Please describe the question(s) you are asking and what you expect to happen (3/4 page limit).

Observational studies in human populations indicate that greater dietary intake of vitamin D, self-reported use of vitamin D supplements, and higher concentrations of serum 25-hydroxy vitamin D (25-OH D) are each protective against several types of cancer. Studies in animal models support this concept, implicating a true causal role for vitamin D. However, there is a paucity of human intervention data and thus the potential utility of incorporating vitamin D in cancer prevention strategies remains an unsettled issue. Moreover, if vitamin D genuinely has a chemoprotective role against cancer development, it is not known whether the benefit is confined only to those whose baseline vitamin D status is inadequate, or whether supplementation of a population that is generally replete will similarly convey protection.

The purpose of the proposed ancillary study is to generate important prospective, placebo-controlled, interventional evidence. Definitive cancer prevention trials in which cancer occurence is the primary endpoint are extraordinarily lengthy and expensive undertakings, and funding can only be secured nowadays when exploratory trials that examine intermediary biomarkers (which precede and predict cancer) have shown efficacy. Regardless of whether a cancer prevention trial is an exploratory one that utilizes intermediary biomarkers or a definitive one that examines cancer occurrence, the design is most effective when the population to be studied is at increased risk of the cancers of interest. Since overweightness and obesity are accepted risk factors for our three cancers of interest, we feel that the D2d study population is a highly suitable one since entry criteria dictate a BMI of 25 or greater.

Our overarching question therefore, is whether vitamin D supplementation over a period of 1-3 years will attenuate intermediary biomarkers that predict the subsequent occurence of lymphoma, breast and colon cancer. A major issue in vitamin supplementation trials of all types is whether a benefit is realized by vitamin-replete individuals as well as those who are deficient: the very nature of the D2d study therefore enables us to also ask the question as to whether supplementation of a generally replete pre-diabetic population experiences a decline in intermediary biomarkers of cancer. We postulate that daily supplementation with 4000 I.U. of vitamin D3 over a period of 1-3 years will significantly alter several intermediary biomarkers of these cancers in a beneficial direction, thereby providing the rationale for a definitive trial in which cancer incidence would serve as the primary endpoint.

To address this hypothesis we propose to conduct a study with the following three specific aims:

1. Determine whether a daily vitamin D3 supplement significantly reduces plasma insulin growth factor 1: insulin growth factor binding protein 3 molar ratio (=primary outcome), as well as estradiol, estrone, estrone sulfate and testosterone.

>examine endpoints at baseline, 1, and 3 years to determine the onset and persistence of a vitamin D3 effect

2. Determine over a period of three years the extent to which a daily vitamin D3 supplement reduces six pre-specified plasma microRNAs (miRNA) linked to ‘high-risk’ adenomas of the colon.

>examine endpoints at baseline, 1, and 3 years to determine the onset and persistence of a vitamin D3 effect

3. Determine if vitamin D receptor FokI and BsmI single-nucleotide polymorphisms modify the response of the endpoints in Specific Aim 1 or 2 to vitamin D intervention.

1c. Significance and Brief Background

Please describe the scientific relevance (1 page limit).

A robust consensus among analyses of prospective cohort studies indicates an inverse link between habitual intake of vitamin D and the subsequent risk of colorectal cancer (CRC) and breast cancer (1-6), although occasional null studies have appeared (e.g.: 7). In several of the studies of colorectal and breast cancer the magnitude of protection associated with vitamin D is significantly greater when intake from supplements is included (1,3,6), suggesting that the greater amount of vitamin D consumption achieved with supplements conveys a degree of protection beyond that achieved with the vitamin D contained in foodstuffs alone. Consistent with these observations are systematic analyses of prospective cohort studies that demonstrate an incremental decrease in the risk of developing these two cancers with greater concentrations of plasma 25-hydroxyvitamin D (25-OH D, references 8,9). Moreover, a similar inverse relationship between 25-OH D levels and the risk of non-Hodgkins lymphoma has been observed by multiple investigators (10,11).

Whether vitamin D plays a true causal role in the prevention of these cancers, and at what doses, remains unclear. True causality has been implicated by a host of controlled studies in relevant animal models of colon and breast cancer, including some which have utilized xenografts from human cancers (12-15). Importantly, supplemental quantitites of D in these animal models seem to be more effective than if the vitamin is delivered at the basal requirement (12).

Two clinical trials in which D3 and calcium supplements were administered to postmenopausal women have been reported but the results are highly discordant, and therefore the question of whether vitamin D supplementation possesses cancer preventive properties remains entirely unsettled: in one trial of ~1200 postmenopausal women, those receiving 1100 I.U. of D3/1500 mgs of calcium daily for 4 years realized a 75% decline in cancer incidence (the majority of which were breast, colon, and lymphoma/leukemia cases) compared to those receiving placebo (16). In contrast, after 11 years of follow-up no benefits were evident among ~18,000 postmenopausal women in the WHI trial who were randomized to receive 400 I.U. of D3 and 1000 mgs of calcium daily (17). The latter trial has received considerable criticism since its design has been felt by some to have obscured the ability to detect a positive outcome. Moreover, since D3 was co-administered with calcium in both of these trials it is impossible to determine whether the vitamin alone has any cancer preventive properties.

As exemplified by the preceding two studies, the conduct of cancer prevention trials is greatly hampered by two logistical considerations: in order to observe a sizeable impact on cancer occurrence exposure to the intervention must occur over several years or longer, and large subject populations must be studied since the overall incidence of cancers is relatively low in the general population. Nowadays, to justify the great expense and time required for such a trial, it is necessary to: 1) conduct precursor trials of an exploratory nature that utilize intermediary biomarkers of cancer as primary endpoints, and 2) provide evidence of plausible biological mechanisms that explain the salutary effect of the intervention. The ancillary study we propose will fulfill both of these observations, and will therefore provide the observations needed to secure funding for a large scale trial.

Although the portfolio of available intermediary biomarkers of cancer are imperfect and offer little predictive value for individuals, many of them nevertheless possess genuine utilitarian value in studies examining sizeable cohorts. The molar ratio between the most bioactive somatomedin, IGF-1, and its major plasma binding protein, IGF BP3, has repeatedly been linked to the risk of developing obesity-associated cancers, and it constitutes our primary endpoint. In our three cancers of interest, (as well as in advanced colorectal adenomas) the relative risk associated with an elevated IGF-1:IGF-1 BP3 ratio is significantly elevated, and typically found to be 1.3-2.8 (18-21). Similarly, elevated levels of estrone, estrone sulfate, estradiol and testosterone have been observed by several investigators to predict the subsequent occurrence of postmenopausal breast cancer, with relative risks generally in the range of 2.0-4.0 when comparing those individuals in the highest quartile compared to the lowest (22-24). Finally, a burgeoning literature documents the utility of quantitative miRNA measurements in the detection of several early stage cancers (including CRC), as well as pre-cancerous lesions such as adenomatous polyps (e.g.: 25-27). The plasma concentrations of six miRNAs have been convincingly linked to 'high-risk' colorectal adenomas (HRAs) (miR-18a, -21, -29a, -92a, -601, -760), and in human CRC xenografts the suppression of tumorigenesis produced vitamin D was observed to be mediated by a miRNA (28).

1d. Innovation & Impact

Please describe the research innovation and potential impact (1/2 page limit).

In most instances the scientific community accepts a new concept as 'scientific fact' only when a consensus of studies from different investigators--using different approaches--supports the concept. Moreover, the need for such a consensus is substantially greater for those concepts that have a particularly high impact on science and society. The scientific literature is full of instances where an apparent 'breakthrough' was soon found to be non-reproducible by others (e.g.: the recent claim that mouse embryonic cells can be derived simply by acidifying mature adults cells [29]).

The proposed study is highly innovative because it constitutes the first placebo-controlled trial to examine the effects of vitamin D3 supplementation on multiple intermediary biomarkers of cancer in a population at high risk of obesity-promoted cancers. The results of a large D3 supplementation trial in the general population (the 'VITAL' trial), for which cancer incidence is a primary endpoint, will be completed in the next few years (30). This study is scheduled to recruit 5000 subjects in the general population randomized to daily vitamin D3 and 5000 to placebo. Our study will offer an important complement to the results of that trial: 1) by providing plausible biochemical and molecular mechanisms that underlie the clinical endpoints observed by VITAL, 2) by studying a targeted population at particular risk of obesity-associated cancers, and 3) by providing plausible biologic explanations for any chemopreventive properties that are observed, assist in building the necessary consensus of observations. In addition, our results may establish novel paradigms for the pathogenesis of three common cancers, fundamentally advancing our understanding of the biology of lymphoma, CRC, and breast cancer, and from an applied perspective contribute important support for the utility of D3 for cancer chemoprevention.

1e. Research Approach

Please address the following: (1 and 1/2 page limit)

- *Study type (e.g. interventional or observational)*
- *Population and setting (inclusion/exclusion criteria)*
- *Study design (e.g. details of study procedures, outcome assessments, confounders, bias)*
- *Schedule of assessments*
- *Sample size (power) calculations*
- *Data analysis plan*

Study type: an ancillary intervention trial that leverages the prospective, randomized, placebo-controlled design of D2d.

Population and setting: the inclusion/exclusion criteria will be identical to D2d (except for the sex hormone analyses, which will only be examined in post-menopausal females).

Study design: Matched samples from all 2382 subjects will be measured for IGF-1:IGFBP3 and six miRs at baseline, 1, and 3 years. Plasma estradiol, estrone, estrone sulfate, and testosterone concentrations will be measured at the same three time points, but only for women participants who are post-menopausal at the time of enrollment (onset defined as 'occurring 12 months after the last menstrual period'). All samples will be measured in duplicate, and values averaged. A small sample of DNA from the time of enrollment will be used to genotype each individual for the FokI and BsmI SNPs in the VDR gene.

IGF-1:IGFBP3: As described (31), IGF-1 levels will be measured using a radioimmunoassay (RIA) kit (American Laboratory Products Company, Salem, NH). The samples to be tested are first treated with an acidic solution to strip the IGF-1 from its binding proteins, after which the carrier proteins are saturated with IGF-2. This abolishes any interference in the assay produced by the binding proteins. IGFBP-3 levels will be determined using the “Active” IGFBP-3 immunoradiometric assay kit (Diagnostic Systems Laboratories, Inc., Webster, TX); the calculated sensitivity was 0.5 ng/ml. For both IGF-1 and IGFBP-3, samples will be analyzed in duplicate. The calculation of the molar ratio of IGF-1:IGFBP-3 is based on the fact that 1 ng/mL of IGF-1 = 0.13 nmoL, and 1 ng/mL of IGFBP3 = 0.036 nmoL. Plasma volume required/sample = 100 microliter.

Please continue Research Approach on next page.

Estrone, estrone sulfate, estradiol, testosterone: In brief, samples will be extracted with a mixture of hexane and ethyl acetate (4:1, vol/vol) and applied to a celite column, the steroids are then eluted from the column (celite in ethylene glycol), and the fractions are subjected to radioimmunoassay, as described (32). NOTE: although many large studies have utilized commercial labs to perform these assays (e.g. 32), we plan to recruit a collaborator from one of the participating institutions--in advance of submitting our NIH application--who has longstanding research expertise in studying these hormones since we believe we will obtain more accurate results, and because smaller quantities of plasma will be required. Plasma required for each sample: 750 microl.

Plasma miRNAs: (miR-18a, -21, -29a, -92a, -601, -760). Although detecting the appearance of HRAs is not a feature of this biomarker study, it is worth noting that this lesion will be a common one in the overweight population of D2d: ~8% of subjects would be expected to have pre-existing HRAs at baseline, and an additional 2.9% of the subjects would be expected to evolve a HRA over the 3 years of the trial (33,34). miRNA species will be analyzed by reverse transcriptase PCR in the manner described by Kroh et al (35). All plasma samples will be re-centrifuged since platelet contamination can affect the results (36). Plasma volume required per sample for all six miRs = 400 microl.

We shall also examine two well-described single-nucleotide polymorphisms (SNPs) in the vitamin D receptor that have been repeatedly linked to our three cancers of interest, and which is an effect modifier of the relationship between vitamin D status and cancer risk: FokI and BsmI (37, reviewed in 38). Each subject will be genotyped by PCR analysis for these two SNPs so that we can examine gene:nutrient interactions with the abovementioned endpoints across all three cancers. DNA required per subject = 40 ng.

Sample size estimates and other statistical considerations: IGF-1:IGFBP3 ratio is our primary endpoint since it has been compellingly linked to the risk of all three cancers. Moreover, the entire study population of D2d will be overweight or obese, and an elevated IGF1:IGFBP3 ratio is a leading hypothesis by which obesity is thought to enhance carcinogenesis. Testing for the adequacy of our sample size is therefore based on this endpoint. In a randomized trial of ~600 overweight/obese subjects, the mean reduction of IGF1:IGFBP3 with weekly vitamin D supplementation was 0.02+0.04 units greater than with the placebo (39). If one assumes a similar reduction in the D2d trial with 1190 subjects in each arm, a two-tailed alpha value of 0.05 and a standard deviation of 0.04, JAVA appelet (http://www.stat.uiowa.edu/~rlenth/Power) estimates a 90% power of observing a significant reduction due to the vitamin D intervention.

Since inadequate human data are available, in order to confirm the sample size estimate in regard to the sex hormone endpoints, we have chosen a study in which supplemental D3 was administered to nude mice that had received xenografts of human breast cancer cells (12). In the ovariectomized mice (model for the post-menopausal state), the tumor estradiol level in the mice receiving 5X the basal requirement of D3 was 22 +4.5 pg/mg protein compared to 85+8.9 in the mice receiving the control diet. If one assumes a similar reduction in the serum of the postmenopausal women in the D2d trial with 200 subjects in each arm, a two-tailed alpha value of 0.05 and a mean standard deviation of 6.7 JAVA appelet estimates a >90% power of observing a significant reduction due to the vitamin D intervention. This assumes that about one-half of the D2d study population will be women and that approximately one-third of the women will be post-menopausal.

To our knowledge, the only data available on the effect of vitamin D on miRNA levels in either humans or animals is one in which an unrelated miRNA was examined in an animal model of colorectal cancer and in which the form of vitamin D was 1,25 dihydroxyvitamin D (28): we have therefore deemed this study to be unsuitable for a estimating sample size for our ancillary study (although, parenthetically, the magnitude of the effect was ~2.5 fold). This ancillary study should therefore be considered exploratory in regard to the miRNA endpoints, although as described above, since the sample size is amply adequate for our other endpoints, there is no harm, and potentially great insight, to be gained by including an exploratory arm.

We shall examine for significant effects of D3 for each endpoint at 12 months using an analysis of covariance adjusted for baseline, and then determine whether the effect is still present at 36 months with ANCOVA as well. Interactions between the presence of either SNP and the effect of D3 will be determined by calculating the difference in a mean quantitative response between alleles, using ANOVA.

1f. References Cited
Please attach a list of references (PDF format document) with the application.

Part 2: Description of Data

2a. Required Sources of Data
Please select all that apply:

- ☑ Existing data collected as part of D2d study
  If yes, please complete ***D2d Ancillary Study Data Request Form***
- ☑ New data derived through use of stored biological specimens collected as part of D2d study
  If yes, please complete ***D2d Ancillary Study Specimen Request Form***
- ☐ New data derived through direct contact with D2d participants (e.g. procedure, survey, observation)
  If yes, please complete question *2b*
  If available, please include a copy of the proposed protocol with your application.

2b. New Data Acquisition, *if applicable*
Please describe the additional procedures, interventions or surveys required for new data acquisition and address the need for additional visits and/or the prolongation of existing visits. (1/2 page limit).

Not applicable.

Part 3: Facilities & Resources

3a. D2d Collaborating Clinical Sites

Please note: By marking the box next to a site(s), the ancillary study PI has secured the commitment of the site(s) to the proposed ancillary study to allow generation of new data by direct contact with D2d participants at the site.

Please select all D2d collaborating clinical sites that have agreed to participate in the proposed Ancillary Study.

- ☐ Atlanta VA Medical Center
- ☐ Baylor College of Medicine
- ☐ Beth Israel Medical Center
- ☐ Duke University Medical Center
- ☐ Florida Hospital Translational Research Institute
- ☐ HealthPartners Research Foundation
- ☐ Los Angeles Roybal
- ☐ Maine Medical Center Research Institute
- ☐ Medical University of South Carolina
- ☐ MedStar Health Research Institute
- ☐ NIDDK Phoenix
- ☐ Northwestern University
- ☐ Pennington Biomedical Research Center
- ☐ Stanford University Medical Center
- ☐ Tufts Medical Center
- ☐ Tulane University Health Sciences
- ☐ University of Kansas Medical Center
- ☐ University of Nebraska Medical Center
- ☐ University of Tennessee Health Science Center
- ☐ University of Texas Southwestern Medical Center

3b. Description of Clinical Laboratory Facilities, *if applicable*

Please describe clinical laboratory facilities and where and how bio-specimens will be handled (e.g. storage, shipping of biological material; laboratory staff experience).

Dr. Mason has been Director of the Vitamins and Carcinogenesis Laboratory in the HNRCA since it was created in 2002, and before that he directed the Vitamins and Carcinogenesis Program in the HNRCA for over a decade. The laboratory is a ~850 square foot facility that is fully equipped for a wide variety of biochemical and molecular analyses. Included among the laboratory's equipment is an ABI 7300, which is a real-time PCR station that is highly suited to quantitative RT-PCR. We therefore plan to have Mason's lab do the miRNA analyses. Although the IGF-1, IGF BP3, and sex hormone assays are available through commercial labs (such as Quest Diagnostics), we plan to secure collaborations with investigators at other D2d sites who are established experts with these particular endpoints in preparation for submission of an NIH grant since we feel that would provide us with more accurate data.

Part 4: Potential Burden on the D2d study

*Please describe the potential burden of the ancillary study **on the D2d study** in relation to the following and provide ways to minimize the burden:*

4a. Participant Burden and Potential for Compromising Participant Retention

Since only stored blood will be used, there will be no burden to participants.

4b. Participant Safety and Confidentiality

Please describe measures taken to ensure participant safety and confidentiality and address plan for data management (secure storage, monitoring etc.).

Dr. Mason's laboratory, and any other laboratory handling the analyses described in this application will not have access to personal identifying information for the subjects since the D2d central repository will de-identify all plasma and DNA samples by assigning them a numerical code.

4c. Burden on Collaborating Clinical Sites

Since only stored blood will be used, there will be no burden to collaborating clinical sites, as specimen and data acquisition will be coordinated via the D2d CC.

4d. Regulatory Requirements

Please describe how informed consent will be obtained and describe plan for local IRB approval. If available, please attach a copy of the informed consent form.

Since no information beyond that already being collected for the parent study is needed, no additional informed consent will be obtained.

Part 5: D2d Support

Please note: The costs associated with the use of resources (D2d or other) must be included in the plans for funding the ancillary study. All negotiated services must be documented in a letter of commitment from the provider of such services.

Please select resources at the D2d Coordinating Center or D2d Central Laboratory that will be required. There will be a fee associated with these services:

- ☑ Data extraction and transfer
- ☑ Specimen selection and transfer
- ☑ Data analyses *(depending upon staff availability, proposed analyses may be conducted by the analytical team at the D2d Coordinating Center*)
- ☐ Other study-specific services for studies that will collect new data in real time

Part 6: Funding

6a. Funding Source(s)

Please describe ancillary study funding source(s) or plans to apply for funding.

The study will seek NIH funding

6b. Planned Date of Submission to Funding Agency 06/05/2014

Part 7: NIH Biosketch

*Please attach NIH Biosketches **for PI only** with your application upon submission.*

D2d Ancillary Study Application

Part 8: Acknowledgement of D2d Ancillary Studies Policies & Procedures

I have read and agree to abide by the policies and procedures for D2d Ancillary Studies as described in the document titled: *D2d Ancillary Studies Policies and Procedures & Instructions for Submission of Proposals*, and specifically regarding the presentation and publication of ancillary study results and data sharing policies.

Principal Investigator Signature _____ Date _____
(e-signature accepted)

Part 9: Attachments

Please indicate all documents that are included with your application:

- ☑ NIH Biosketches for PI only ***(required for all applications)***
- ☑ References Cited ***(required for all applications)***
- ☑ D2d Ancillary Study Data Request Form
- ☑ D2d Ancillary Study Specimen Request Form

Please note: The following forms are not required with application, but are required *prior to study initiation:*

- ☐ Ancillary Study Proposal
- ☐ Ancillary Study Manual of Procedures
- ☐ Informed Consent Form *(required for studies involving generation of new data via direct contact with D2d participants)*
- ☐ IRB Approval Letter

Final steps to submission:
Save a copy of this form to your computer.
Click on the "Submit" button, which will open an email and automatically attach application. In that email, attach additional documents checked above to complete application then send.

Submit

Thank you for your interest in the D2d Study

D2d Ancillary Study Application

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Principal Investigator Signature *(e-signature accepted)* _____ Date 4/8/14

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Click on the "Submit" button, which will open an email and automatically attach application. In that email, attach additional documents checked above to complete application then send.

Submit

Thank you for your interest in the D2d Study

D2d Ancillary Study Data Request Form

Office Use
Ancillary Study Number AS14-01 Date Submitted (MM/DD/YYYY) 04/08/2014

Title of Proposal
(81 character limit)
Biomarkers of cancer in the D2d trial

Principal Investigator Joel B. Mason, M.D.
Institutional Affiliation U.S.D.A. Human Nutrition Research Center at Tufts University

1. Data Requested

Please indicate required time assessments for each outcome category requested.

Outcome Category	Time Assessed									End of study	
	Base	M03	M06	M12	M18	M24	M30	M36	M42	M48	Conf*
Medical History	☒	☐	☐	☒	☐	☐	☐	☒	☐	☐	☐
Physical Examination	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Vital Signs	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Waist Circumference	☒	-	-	-	-	-	-	-	-	-	-
Non-Study Medication Review	☒	☐	☐	☒	☐	☐	☐	☒	☐	☐	☐
Food Frequency Questionnaire	☒	-	-	-	-	-	-	-	-	☐	☐
Physical Activity Questionnaire	☐	-	-	☐	-	☐	-	☐	-	☐	-
Study Pill Adherence	☒	-	☐	☒	☐	☐	☐	☒	☐	☐	☐
HbA1c, Fasting Plasma Glucose	☒	-	☐	☒	☐	☐	☐	☒	☐	☐	☐
2-hour Plasma Glucose (OGTT)	☐	-	-	☐	-	☐	-	☐	-	☐	☐
Plasma Glucose after 30 min (OGTT)	☐	-	-	☐	-	☐	-	☐	-	☐	-
25-hydroxyvitamin D	☒	-	-	☒	-	☐	-	☒	-	☐	-
Serum Insulin, fasting	☒	-	-	☒	-	☐	-	☒	-	☐	-
Serum Insulin, after 30 min (OGTT)	☐	-	-	☐	-	☐	-	☐	-	☐	-
Urine Albumin-Creatinine Ratio	☐	-	-	☐	-	☐	-	☐	-	☐	-
Urine Calcium-Creatinine Ratio	☐		-	☐	-	☐	-	☐	-	☐	-

***Conf = a confirmatory visit to confirm the diagnosis of diabetes.**

2. Comments

If necessary, please use the space below to provide additional comments.

Final steps to submission:
Save a copy of this form to your computer.
Attach to D2d Ancillary Study application and submit in a single email to: D2d@TuftsMedicalCenter.org.

D2d Ancillary Study Specimen Request Form

Office Use
Ancillary Study Number AS14-01 Date Submitted (MM/DD/YYYY) 04/08/2014

Title of Proposal
(81 character limit)
Biomarkers of cancer in the D2d trial

Principal Investigator Joel B. Mason, M.D.
Institutional Affiliation U.S.D.A. Human Nutrition Research Center at Tufts University

1. Sample Specifications

Please select all that apply:

Age Range
- (•) All *(30 years and older)*
- ( ) Other, please specify: _____

Sex
- (•) All
- ( ) Female
- ( ) Male

Race
- (•) All
- ( ) White
- ( ) Black
- ( ) Asian
- ( ) Other, please specify: _____

Ethnicity
- (•) All
- ( ) Hispanic
- ( ) Non-Hispanic

D2d Ancillary Study Specimen Request Form

2. Specimens Requested

Please indicate treatment group and time-point (in months, starting with baseline) for each specimen type requested. Specimens not collected at a particular time-point are indicated with a dash.

Please note:

Each vial of **serum or plasma** contains **0.5 mL**
Each vial of **whole blood** contains **2 mL**
Each vial of **urine** contains **1.5 mL**

Specimen Type	Placebo									Vitamin D								
	0	6	12	18	24	30	36	42	48	0	6	12	18	24	30	36	42	48
DNA	☒	-	-	-	-	-	-	-	-	☒	-	-	-	-	-	-	-	-
Whole Blood	☐	-	☐	-	☐	-	☐	-	☐	☐	-	☐	-	☐	-	☐	-	☐
Serum	☐	☐	☐	-	☐	-	☐	-	☐	☐	☐	☐	-	☐	-	☐	-	☐
Plasma	☒	☐	☒	-	☐	-	☒	-	☐	☒	☐	☒	-	☐	-	☒	-	☐
Urine without preservative	☐	-	☐	-	☐	-	☐	-	☐	☐	-	☐	-	☐	-	☐	-	☐
Urine with acid preservative	☐	-	☐	-	☐	-	☐	-	☐	☐	-	☐	-	☐	-	☐	-	☐

Final steps to submission:
Save a copy of this form to your computer.
Attach to D2d Ancillary Study application and submit in a single email to: D2d@TuftsMedicalCenter.org.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Mason, Joel B.	Professor and Senior Scientist
eRA COMMONS USER NAME (credential, e.g., agency login) JMASON	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Illinois, Urbana, IL	BS	1977	
University of Chicago, Chicago, IL	MD	1981	Medicine
University of Iowa Hospitals and Clinics, Iowa City, IA	Internship & Residency	1981-1984	Internal Medicine
University of Chicago Hospitals and Clinics, Chicago, IL	Fellowship	1984-1987	Gastroenterology/Nutrition

A. Personal Statement

From 1990 through 2009 my primary research focus was the relationship between the intake of 1-carbon nutrients (eg: folate, vitamins B2, B6 and B12, and methionine) and the risk of developing cancer of the colorectum. It was my laboratory that took what was merely an epidemiological association in the late 1980s and proved, within the framework of an animal model, a true cause and effect relationship between inadequate folate intake and increased colorectal tumorigenesis. In the ensuing years we built extensively upon this initial foundation, pursuing the mechanistic pathways through which the availability of 1-carbon nutrients modulate carcinogenesis. We were the first to identify, and confirm, how mild inadequacies of these nutrients activate an important pro-transformational cell-signaling pathway in colon, the *Wnt* cascade. Our mechanistic work has also examined a variety of other co-determinants such as age, ethanol consumption, gene variants and availability of the other 1-carbon vitamins in determining the effects of folate status on colorectal carcinogenesis, Over the years, we have emphasized translational and other types of clinical studies as well in order to determine the relevance of our mechanistic observations in animal models to human cancer biology: in this pursuit we have successfully conducted 3 studies within the colonoscopy suite at Tufts Medical Center.

Over the past three years my laboratory has devoted an increasingly larger portion of its efforts towards examining the mechanistic link between obesity and colon carcinogenesis, and this now constitutes the majority effort of my laboratory. We are busily engaged in mechanistic studies in animal models aimed at identifying the biochemical and molecular pathways by which obesity exerts its impact on cancer risk, and we have recently completed a translational study of obese and lean individuals undergoing routine colonoscopic screening.

I have been studying the nutritional modulation of colon carcinogenesis in both animal models and humans for over 20 years, with funding as P.I. from the NCI, NIEHS, AICR, International Life Science Institute, Agricultural Research Service and U.S.D.A. and therefore have both the scientific expertise and management skills necessary to conduct the studies proposed in this application.

B. Positions and Honors

Positions and Employment

1987-1988 Res Associate, JM USDA Human Nutrition Research Center on Aging at Tufts University (HNRCA)
1988-1989 Scientist III, HNRCA
1988-1997 Assistant Professor, Tufts University, Schools of Medicine & Nutrition
1989-1996 Scientist II, HNRCA
1996-2010 Scientist I, HNRCA
1996-present Chief, Vitamins and Carcinogenesis Laboratory, HNRCA
1996-2009 Associate Professor, Tufts University Schools of Medicine & Nutrition

1997-2002 Chief, Division of Clinical Nutrition, Tufts University, School of Medicine
2001 *ad-hoc* member, NCI Scientific Review Group, Subcommittee G
2003-2008 Member, NCI Scientific Review Group, Subcommittee J
2009, '10 *ad hoc* member, NCI Scientific Review Group, CDP
2010, '11 Chair, NIH Special Emphasis Panel, The Role of Microbial Metabolites in Cancer Prevention and Etiology, PAR-10-208
2005-present Associate Director, Tufts Cancer Center
2010-present Professor, Tufts University Schools of Medicine & Nutrition
2011-present Senior Scientist (=Professor), HNRCA

Honors

1976 Phi Beta Kappa, Univ. of Illinois-Urbana,
1981 Kraftco Fellowship Award for Clinical Nutrition Research, University of Chicago,
1990 International Life Science Institute: Future Leader in Nutrition Award
1991 SmithKline/Beecham Award for Clinical Research, American Gastroenterological Association
2004-7 Most highly cited paper in the *Journal of Nutrition* four years in a row (Choi SW, Mason JB. J Nutr 2000;130:129-32)
2007 Most highly cited paper in *Cancer Epidemiol Biomarkers and Prevention* (Mason JB et al. CEBP 2007;16:1-5)
2009 Invited expert on folate and cancer risk, European Union Food Safety Authority, Meeting on "Folic Acid: an update on scientific development," Uppsala, Sweden
2010 Mary Swartz Rose Senior Investigator Award, American Society for Nutrition
2010 Fellow, American Gastroenterological Association
2014 E.V. McCullom Award, American Society for Nutrition

C. Selected Peer-reviewed Publications

Most relevant to the current application

1. Liu Z, Brooks RS, Ciappio ED, Kim SJ, Crott JW, Bennett G, Greenberg AS, and **Mason JB**. Diet-induced obesity elevates TNF-α in mice and is accompanied by an activation of *Wnt* signaling: a mechanism for obesity-associated colorectal cancer. J Nutr Biochem. 2012;23: 1207-13. PMCID in process.
2. Protiva P, **Mason JB**, Liu Z, Hopkins ME, Nelson C, Lambrecht RW, Pendyala S, Marshall JR, Kopelovich L, Kim M, Kleinstein S, Laird PW, Lipkin M, Holt PR. Altered folate availability modifies the molecular environment of the human colon: implications for colorectal carcinogenesis. Cancer Prev Res 2011;4: 530-43. PMCID 21321062
3. Flood A, **Mason JB**, Zhenhua L, Cash BD, Schatzkin A, Schoenfeld PS, Cross AJ. Concentration of folate in colorectal tissue biopsies predicts prevalence of adenomatous polyps. Gut. 2011;60: 66-72. PMCID 21068136
4. Liu Z, Ciappio E, Crott J, Brooks R, Nesvet J, Smith D, Choi SW, **Mason JB**. Combined inadequacies of multiple B-vitamins amplify colonic *Wnt*-signaling and promote intestinal tumorigenesis in *BAT-LacZ×Apc1638N* mice. FASEB J. 2011;25: 313-46. PMCID: PMC3157689
5. Kim Y-I, Baik H, Fawaz K, Knox T, Lee Y-M, Norton R, Libby E, **Mason JB**. Effects of folate supplementation on two provisional molecular markers of colon cancer: a prospective, randomized trial. Am J Gastro 2001;96: 184-95.

Additional recent publications of importance to the field

1. Byun AJ, Hung KE, Fleet JC, Bronson RT, **Mason JB**, Garcia PE, Crott JW. Colon-specific tumorigenesis in mice driven by Cre-mediated inactivation of Apc and activation of mutant Kras. Cancer Letters March 2014 (epub ahead of print). PMID 24632531.
2. Selhub J, Byun A, Liu Z, **Mason JB**, Bronson RT, Crott JW. Dietary vitamin B6 intake modulates colonic inflammation in the IL10-/- model of inflammatory bowel disease. J Nutr Biochem. 2013;24: 2138-43. PMID: 24183308.
3. Ciappio E, Liu Z, Brooks R, **Mason JB**, Bronson R, Crott J. Maternal B-vitamin supplementation from preconception through weaning suppresses intestinal tumorigenesis in *Apc*$^{+/1638N}$ mouse offspring. GUT. 2011;60: 1695-702. PMID: 21659408.

4. Tao MH, **Mason JB**, Marian C, McCann SE, Platek ME, Millen A, Ambrosone C, Edge SB, Krishnan SS, Trevisan M, Shields PG, Freudenheim JL. Promoter methylation of E-cadherin, p16, and RAR-β(2) genes in breast tumors and dietary intake of nutrients important in one-carbon metabolism. Nutr Cancer. 2011;63: 1143-50.
5. Tomaszewski JJ, Cummings JL, Parwani AV, Dhir R, **Mason JB**, Nelson JB, Bacich DJ, O'Keefe DS. Increased cancer cell proliferation in prostate cancer patients with high levels of serum folate. Prostate 2011;71: 1287-93. PMCID: PMC3120927
6. Marian C, Tao M, **Mason JB**, Goerlitz DS, Nie J, Chanson A, Freudenheim JL, Shields PG. Single nucleotide polymorphisms in uracil-processing genes, intake of one-carbon nutrients and breast cancer risk. Eur J Clin Nutr. 2011;65: 683-9. Epub 2011 Mar 23. PMID: 21427733
7. Chanson A, Parnell LD, Ciappio ED, Liu Z, Crott JW, Tucker KL, **Mason JB**. Polymorphisms in uracil-processing genes, but not one-carbon nutrients, are associated with altered DNA uracil concentrations in an urban Puerto Rican population. Am J Clin Nutr 2009;89:1927-36. PMCID: PMC2683003.
8. **Mason JB**, Dickstein A, Jacques P, Haggarty P, Selhub J, Dallal G, Rosenberg IH. A temporal association between folic acid fortification and a rise in colorectal cancer rates may be illuminating important biological principles: a hypothesis. Cancer Epidemiol Biomarkers Prevent 2007;16: 1-5
9. van den Donk M, Pellis L, Crott J, van Engeland M, Friederich P, Vallei Ede G, Y. de Boer S, Nagengast F, **Mason JB**, Kok F, Keijer J, Kampman E. Supplementation with folic acid in combination with vitamin B-12 does not favorably influence uracil incorporation and promotor methylation in rectal mucosa DNA among those with previous colorectal adenomas. J Nutr 2007;137: 2114-20.
10. Gabriel HE, Crott JW, Ghandour H, Dallal GE, Choi SW, Keyes MK, Jang H, Liu Z, Nadeau M, Johnston A, Mager D, **Mason JB**. Chronic cigarette smoking is associated with diminished folate status, altered folate form distribution and increased genetic damage in the buccal mucosa of healthy adults. Am J Clin Nutr 2006;83: 835-41.

D. Research Support

Ongoing

1950-5100-074-01S (PI: Mason JB) 10/01/09-09/30/14
USDA/ARS (10/1/15-9/30/20 renewal pending)
Nutrition and Cancer Prevention
This cooperative agreement provides the core support for the Vitamins and Carcinogenesis Laboratory, the major goals of which are to elucidate the means by which: 1) obesity and 2) 1-carbon nutrients modulate the risk of cancer development and to translate such knowledge into tools for cancer chemoprevention.

Prevent Cancer Foundation (PI: Mason JB) 02/01/13-1/31/15
The major goals of these studies in mice are to define the relative contributions of IL-1β and TNF-α in mediating the promotional effects of diet-induced obesity on colon carcinogenesis.

R01 CA13844404-04 (PI: O'Keefe DS) 02/01/10-01/31/15
NIH/NCI
Folate and PSMA Interact to Regulate DNA Methylation and Prostate Carcinogenesis
The studies use an animal model to examine how a prostate protein, PSMA, acts as a folate transporter in the prostate, and how the level of dietary folate impacts on DNA methylation in prostate cancer development.
Role: Co-Investigator

R01 DK073321-05 (PI: Lichtenstein AH) 09/01/07-08/31/13 (NCE)
NIH/NIDDK
Evaluation of Glycemic Index to Assess Diet-Associated Chronic Disease Risk
The objective is to assess the validly of using glycemic index and glycemic load as criterion with which to make chronic disease (obesity, heart disease, diabetes) risk reduction recommendations.
Role: Study Physician

HNRCA Pilot Program (PI: Mason JB) 04/01/11-09/30/14 (NCE)
Translational study of TNF-α-mediated *Wnt*-signaling as a molecular mechanism for obesity-associated colorectal carcinogenesis

The objective of this project is to determine whether the concept of obesity-induced inflammation as a promoter of colorectal carcinogenesis is an operable pathway in the human.

Completed

R21 CA150118-02 (PI: Mason JB) 02/01/11-01/31/14 (NCE)
NIH/NCI
Defining the promoting effect of folate on colorectal cancer in a novel animal model
This proposal will utilize a novel animal mouse model of colorectal carcinogenesis to monitor the development of colonic neoplasms *in vivo* in order to determine whether the many sources of folic acid that supplement the quantities naturally present in foods are collectively sufficient to produce the cancer-promoting effect of folate.

R21 ES019102 (PI: Mason JB) 09/30/09-03/31/12
NIH/NCI
The MTHFR C677T SNP exerts bipolar effects on colorectal cancer risk through the Wnt pathway
The purpose of this proposed animal study is to define the mechanistic basis for the interaction between the common genetic variant, C677T, and the availability of folate and other related B-vitamins in determining the risk of colon cancer.

R01 AG025834-05 (PI: Choi SW) 03/01/07-02/28/13 (NCE)
NIH/NIA
Effects of aging and folate on colonic carcinogenesis
A series of rodent studies will be performed to define how elder age and folate metabolism interact mechanistically to determine the risk of colorectal cancer.
Role: Co-Investigator

2009-35200-05016 (PI: Mason JB) 12/01/08-11/30/10
USDA
Interaction between one-carbon nutrient status and polymorphisms in uracil repair genes in determining DNA stability
The goal of this project is to determine whether folate and other one-carbon nutrient status affects levels of uracil in the human genome, and how relatively common polymorphisms in uracil-repair genes modify these associations. This project will also aim to determine whether uracil levels in blood DNA may be used as a surrogate measure of uracil levels in breast DNA.

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