ARIC Manuscript Proposal #2187

PC Reviewed: 8/13/13	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Circulating beta-2 microglobulin (B2M) and cancer risk and mortality: Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): Serum B2M and cancer

2. Writing Group: Anna Prizment, Amy Linabery, Pamela Lutsey, Heather Nelson, Aaron Folsom, Elizabeth Platz, Corinne Joshu, Elizabeth Selvin; others welcome

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. _AP_ **[please confirm with your initials electronically or in writing]**

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ARIC author to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).

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3. Timeline: The analysis will be begin as soon as the proposal is approved

4. Rationale:

Beta-2 microglobulin (B2M) is a subunit of the major histocompatibility complex (MHC) class I molecule present on all nucleated cells and thrombocytes[1]. It is especially abundant in immunocompetent cells, such as lymphocytes and monocytes[2]. The synthesis of B2M is regulated by interferons and proinflammatory monocytic cytokines[3,4]. Under physiological conditions, it is present at low levels in serum, urine and other body fluids[5]. B2M is generated at a constant rate and is eliminated by the kidneys. Elevated B2M levels are observed in many diseases, including renal disease[6] immunodeficiency, autoimmune[1,2], and inflammatory bowel diseases[7]. Several cohort studies, including the ARIC study[6], have reported a positive association between B2M and all-cause, cardiovascular, and diabetes mortality[2,6,8,9]. Serum B2M was shown to better predict mortality than other inflammatory markers such as C-reactive protein[2].

Many studies have demonstrated that serum or urine B2M concentration is increased at the time of diagnosis or thereafter in a variety of abnormal growth diseases including B-cell leukemia, lymphomas and multiple myeloma[10,11]. In the lymphoid malignancies, the B2M level serves as an independent and significant prognostic factor. Several studies have also shown increased circulating B2M in patients with solid tumors: breast, prostate, lung, renal, gastrointestinal and nasopharyngeal cancers[5,11-13]. Unexpectedly, studies on the progression of colorectal cancer (especially colorectal tumors with microsatellite instability) suggest that high B2M expression in colorectal tumor tissues is associated with absence of metastasis, disease relapse, and favorable outcome[5,14-17]. To our knowledge, all of the studies on this topic have examined B2M levels after cancer diagnosis.

Recent studies have suggested that B2M is expressed in different ways in normal and tumor cells and the impact of B2M could vary at various stages of cancer development[11]. In spite of this, there have been no studies examining circulating B2M in relation to cancer risk. Likewise, mechanisms explaining the associations between B2M and the development of malignant diseases have not been previously examined, but given its diverse functions, B2M could both promote (e.g., as an inflammatory marker or growth stimulating factor that activates tumor cell growth, epithelial mesenchymal transition, systemic spread, and bone metastasis) and inhibit tumor development (apoptosis-inducing)[5,11]. We hypothesize that in healthy people, an increase in B2M reflects an ongoing low-grade inflammation which starts as the activation of macrophages and T-lymphocytes and leads to the secretion of cytokines, followed by the chronic activation of other types of inflammatory cells[11]. One possible explanation for an inverse association with colorectal cancer progression is that a decreased expression of B2M leads to the loss of MHC class I-mediated antigen presentation, allowing tumor cells to evade immune destruction by cytotoxic T cells[11].

5. Main Hypothesis/Study Questions:

- Is serum B2M measured prior to cancer detection associated with increased cancer risk and mortality from cancer? We are planning to examine all cancers combined, the four most common solid cancers (colorectal, lung, prostate, and breast cancers) individually, and hematological cancers combined.
- Are the observed associations independent of other inflammatory risk factors (CRP, white blood cell counts, fibrinogen)?

Our strongest hypothesis is that B2M is positively associated with the risk and mortality of:

- colorectal cancer since this is the cancer most consistently associated with inflammation and
- hematological cancers (combined) due to the established role of B2M in the progression of myeloma and lymphoma.

Currently, incident cancer cases have been ascertained until 2006 in the ARIC cohort. First, we are planning to conduct an exploratory analysis and publish a brief report of the association of B2M and cancer incidence and mortality (total, four most prevalent cancers, and hematological cancers combined). We are unaware of any previous studies on this topic. If our hypotheses are confirmed, our writing group will submit additional proposals in order to examine each of the cancer-specific associations in more detail (e.g., by stage, subsite for colorectal cancer) and the survival of cancer patients after the new linkage is complete. Since there will be many more cases after the completion of linkage, in the future, we will examine:

- SNPs that were shown to be associated with B2M level in the ARIC GWAS[18] in relation to cancer risk;
- Survival of cancer patients in relation to circulating B2M and
- Interaction with BMI, smoking, and hormone therapy in great detail.

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

Study design: Prospective cohort of all ARIC participants without prevalent cancer at Visit 2.

B2M, CRP, Cystatin C, and creatinine were measured in 2012-2013 in frozen serum in all ARIC participants with available stored serum samples as part of Liz Selvin's Ancillary Study. Thus, Visit 2 will serve as the baseline for this analysis.

Inclusion/Exclusion into the analytic cohort:

- Exclude individuals diagnosed with cancer prior to Visit 2
- Exclude individuals for whom B2M measurement is missing
- Include only white or black; black in Jackson, white in Minneapolis and Washington County

Independent variables: Serum B2M was measured at ARIC Visit 2 for all the participants.

B2M was measured in serum using Roche β2M reagent on the Roche Modular P Chemistry analyzer. Latex-bound anti-β2-microglobulin antibodies react with antigen from the sample to form antigen/antibody complexes which are determined turbidimetrically after agglutination. The laboratory inter-assay CV was 7.3% at a value of 1.58 mg/L and 5.9% at a value of 0.66 mg/L.

Dependent variables: During 1990-2006, there were ~2600 incident total cancers ascertained, including 270 colorectal, 380 post-menopausal breast, 570 prostate, 350 lung and 190 (combined) hematological cancers. Hematological cancers will be examined because the strongest association between B2M and cancer progression has been observed for myeloma and

lymphoma. In addition, we will examine an association of B2M with total and cancer-specific mortality.

Other covariates (Visit 2 unless otherwise noted) include age, race, study site, sex, education, physical activity, reproductive factors (age at menarche, number of live births) in the analysis of female breast cancer, blood fibrinogen, (all measured at Visit 1), smoking status and number of smoking-years, age at menopause and use of post-menopausal hormones (current/former/never use) for women, aspirin use, white blood cell count, C-reactive protein, serum creatinine, and cystatin C.

Analysis: Baseline characteristics of participants will be examined across quartiles of B2M using chi-square tests or proc glm. We will use Cox proportional hazards regression to estimate the age and multivariable hazard ratios (and 95% confidence intervals) for cancer incidence and mortality (overall and by cancer type) in relation to B2M.We will examine all the associations in the whole cohort and stratified by sex. Participants will start contributing time at risk at the second visit (1990-1992) with time since Visit 2 as the time metric. Time to cancer incidence will be calculated as the time from baseline to December 31, 2006, the date of the first primary cancer diagnosis, death or loss to follow-up, whichever occurred first. Time to cancer mortality will be calculated as the time from baseline to December 31, 2009, death or loss to follow-up, whichever occurred first. Cubic splines will be used to test for non-linearity, and guide decisions regarding the correct way to model B2M. Tentatively, we anticipate that B2M will be examined as quartiles and as a continuous variable. The proportional hazards assumption will be tested by graphing the log(-log(survival)) versus log(time). For all observed associations, we will examine whether they persist after excluding the first 2-5 years of follow-up in order to allow for a latency period. Interactions with age, sex, smoking, and BMI will be examined when power allows.

All the variables ("other covariates listed above") will be tested as potential confounders. We will adjust for other markers of kidney function such as serum creatinine and cystatin C, given that B2M is a biomarker of kidney function and several studies suggested that (1) kidney dysfunction is associated with total and specific cancer subtypes[19-21] and (2) cystatin C may be correlated with poor prognosis in the progression of several cancers – breast, ovarian, gastrointestinal cancers and multiple myeloma[22-25]. To test an independent role of kidney function on cancer development, we will also examine an association between creatinine and cystatin C and cancer risk and mortality in an additional analysis.

7.a. Will the data be used for non-CVD analysis in this manuscript? X Yes ____ No

b. If Yes, is the author aware that the file ICTDER03 must be used to exclude persons with a value RES_OTH = "CVD Research" for non-DNA analysis, and for DNA analysis RES_DNA = "CVD Research" would be used? X Yes ____ No
(This file ICTDER has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript? ____ Yes X No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to exclude those with value RES_DNA = "No use/storage DNA"? **____ Yes ____ No**

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.cscc.unc.edu/ARIC/search.php **X Yes ____No**

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? **X Yes ____ No**

11.b. If yes, is the proposal

_X_ A. primarily the result of an ancillary study (list number* 1995.04, 2009.16 (PI: Selvin)

___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* _____ _____ _____)

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

12a. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is **your responsibility to upload manuscripts to PUBMED Central** whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in http://www.cscc.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to Pubmed central.

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