ARIC Manuscript Proposal #3379

PC Reviewed: 5/14/19 Status: _____ Priority: 2
SC Reviewed: _________ Status: _____ Priority: _____

1.a. Full Title:
Determine whether adults with genetic patterns that lead to high levels of IL-1β have an increased risk for developing cancers

b. Abbreviated Title (Length 26 characters): IL-1β and Cancer Risk

2. Writing Group:
Writing group members:
Ken Kornman
University of Michigan

Gordon W Duff
St Hilda’s College, University of Oxford

Lynn Doucette-Stamm

James Beck
University of North Carolina

Kevin Moss
University of North Carolina

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

First author:
Address: Ken Kornman
Department of Periodontics and Oral Medicine
University of Michigan School of Dentistry
1011 N. University Ave.
Ann Arbor, MI 48109-1078 USA

Phone: 617-480-8332 Fax:
E-mail: kkornman@umich.edu

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).
3. **Timeline**: 6 months for data analysis then 6 additional months for Manuscript Preparation

4. **Rationale**:

Inflammation is a protective response to any challenge from external pathogens and danger signals, including ultraviolet radiation, or cells in the body that have become damaged or have surface markers that alter or subvert the host clearance systems, as in tumor cells. Controlled inflammation is the key to host defenses, but inflammation is damaging if prolonged and not permitted to resolve appropriately or if the inflammatory response is too vigorous. Chronic low-grade systemic inflammation, as measured by blood levels of high sensitivity C-reactive protein (hsCRP), is a major factor in first and 2nd myocardial infarctions and strokes. Drugs that lower inflammation, as assessed by hsCRP, result in fewer 1st and second heart attacks and strokes during a two to four-year monitoring period.\(^1\)\(^-\)\(^3\) The influence of elevated low-grade systemic inflammation in many common diseases such as cardiovascular disease is now generally accepted,\(^4\) and the recent results of Novartis’ CANTOS Study serve as a strong proof of principle as to the critical opportunity to control some chronic diseases by controlling chronic inflammation.\(^5\)

In CANTOS, patients with a prior history of myocardial infarction and had hsCRP $\geq 2$mg/L were randomized to a potent IL-1 blocking drug (canakinumab) or placebo. The IL-1 blocking drug is very specific for lowering interleukin-1β (IL-1β) driven inflammation, which it did in this study. Patients were monitored for recurrent major cardiovascular events over the subsequent 3 to 4 years. Patients randomized to canakinumab had 15% to 25% fewer events than those on the placebo.\(^5\), \(^6\) In addition, a predefined secondary outcome of the trial was incidence of lung cancers. Patients on canakinumab that lowered IL-1 driven inflammation developed 70% fewer cases of lung cancer than those on placebo.\(^5\), \(^6\)

**Periodontitis and cancer risk in the ARIC study**

Recently, a group of ARIC investigators (JDB, SO) reported\(^8\) that individuals with clinically assessed severe periodontitis exhibited an increased risk of incident total cancer (HR= 1.24; 95% CI = 1.07 to 1.44; p-trend= 0.004) when compared to individuals with no to mild periodontitis. The ARIC patients were followed for 14.7 years, with incident cancers and cancer deaths used as the primary outcomes. Strong associations were observed for severe periodontitis and lung cancer (HR equals 2.33, 95% CI = 1.51 to 3.60; p-trend <0.001), with other risk factors including smoking, gender, race, and T2D control. In addition, although there were only 48 pancreatic cancers observed, there was a higher risk among individuals with severe periodontitis compared to no or mild periodontitis (HR = 1.65; 95% CI equals 0.72 to 3.77), with the association stronger among whites.

**IL-1β is strongly implicated in severe and progressive periodontitis**

IL-1β levels in gingival crevicular fluid (GCF) and saliva are among the strongest indicators of severe and progressive periodontitis,\(^9\)\(^-\)\(^14\) and IL-1β gene expression in gingival tissue is increased during initiation and progression of periodontitis.\(^15\) IL-1β activates osteoclastic bone loss and
matrix-metalloproteinases which are characteristic of chronic periodontitis. In addition, treatment with a human soluble IL-1 receptor type I as a highly specific inhibitor of IL-1 biologic activity, reduced periodontal bone and connective tissue loss by 60-70% in a high bacterial challenge *Macaca fascicularis* primate model of periodontitis.

**IL-1 and IL-37 genetic patterns that identify high producers of IL-1β**

During the past 25 years research teams throughout the world reported that some individuals consistently produced high levels of IL-1β while others produced lower levels. During that period of time, led by Gordon Duff and his colleagues at the University of Sheffield, a group of investigators on this proposed project collaborated several times to take newly sequenced results from the IL1 gene cluster on chromosome 2q13 to identify a set of biologically functional variations in the promoter region of the IL1B gene. The investigators including Gordon Duff’s group at the University of Sheffield, Genome Therapeutics and Interleukin Genetics both based in Boston, and a team led by Stephen Offenbacher and Jim Beck at the University of North Carolina in Chapel Hill collaborated to report that four SNPs in the promoter region of the IL1B gene exhibited allele specific differential binding of transcription factors and differences in gene transcription that were dependent upon haplotype context. The group went on to demonstrate that individuals with specific patterns of variants in the IL1B gene produced 2 to 3 times more IL-1β protein from stimulated blood mononuclear cells. This group also reported that individuals with genotype patterns consistent with higher blood mononuclear cell expression of IL-1β protein also had 28 to 50% higher tissue levels of IL-1β, as measured in gingival fluid.

Using refined versions of the IL1 genotype patterns we reported 2 prospective studies of recurrent major cardiovascular events in which elevated levels of lipoprotein(a) were shown to predict events but that prediction was conditional on the patient carrying one of the high producing IL1B genetic patterns. Recently, investigators at the University of North Carolina reported the association of IL-37 variants with high concentrations of IL-1β measured in the gingival crevicular fluid (GCF). A recently published manuscript reported the identification of two missense polymorphic variants in the anti-inflammatory IL37 gene locus showed to be associated with high IL-1β levels detected in GCF of subjects with chronic periodontal disease.

**IL37 expression and its role in cancers**

Chronic inflammation is one of the characteristics of cancer and IL-37 might inhibit cancer development through suppression of inflammation pathways. In a review paper, Ding VA and co-authors have discussed the IL37 anti-inflammatory characteristics both in innate immune responses as well as in acquired immune responses by downregulating pro-inflammatory molecules.

Through *in vitro* and xenograft models aiming to investigate the effects of exogenous IL-37 on the biological characteristics of human lung adenocarcinoma A549 cells, Chen YH, et al. found that exogenous IL-37 could inhibit the proliferation, migration and invasion of human lung adenocarcinoma cells (A549) as well as the chemotaxis of regulatory T cells, while promoting the apoptosis of A549 cells. IL-37 was also shown to have an inhibitory role in colon cancer development and function, mainly via β-catenin suppression. The authors discussed IL-37 findings as a novel prognostic indicator and a promising therapeutic target.

**5. Main Hypothesis/Study Questions:**

(Please note: There is overlap with Manuscript Proposal #3115. We have discussed this with Elizabeth Platz and are in agreement on how to make this proposal and #3115 be complementary to each other.)

a. Elevated GCF levels of IL-1β increase the risk of incident total cancers or total cancer related deaths.

b. Elevated GCF levels of IL-1β increase the risk of incident lung cancers or total lung cancer related deaths.

c. IL-1 genetic patterns or IL-37 variants that have been shown to identify high producers of IL-1β increase the risk of incident lung cancers or total lung cancer related deaths.

d. IL-1 genetic patterns or IL-37 variants that have been shown to identify high producers of IL-1β increase the risk of incident total cancers or total cancer related deaths.
Secondary hypothesis:
  a. ARIC subjects with elevated lipoprotein(a) increased the risk of incident total cancers or total cancer related deaths conditional on the individuals being high producers of IL-1β as stratified by IL-1 genetic patterns or IL 37 genetic patterns.

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).

Analytic population: Men and women who self-reported being edentulous at Visit 4 or who attended the clinical dental examination at Visit 4, who did not have a history of cancer by Visit 4, and who consented to genetic studies and studies on chronic diseases including cancer.

Outcomes:
  1. Primary outcome: Total incident cancers plus cancer deaths
  2. Secondary outcomes:
     a. Incident lung cancers plus lung cancer deaths
     b. Incident pancreatic cancers plus pancreatic cancer deaths
     c. Incident colorectal cancers plus colorectal cancer deaths

Exposures:
Main Hypotheses a, b, c.
We will classify participants using:
  1. GCF IL-1β protein level above vs below median, and highest vs lowest tertiles
  2. IL-1 genetic patterns associated with high expression of IL-1β vs patterns associated with lower expression using.
     a. IL-1 genotype patterns
     b. IL-37 genotype patterns

Outcome: We will use the ARIC cancer case files, which were developed using data from the MN, NC, MD, and MS state cancer registries, medical records, and hospital discharge codes.
Other variables: Age, race, Gender, BMI, current smoking status and packyears smoked at Visit 4; alcohol drinking at Visit 4 (never, former, or current drinker), diabetes status at visit 4 ever use of hormone replacement therapy (women only; Visits 4). Ancestry PC’s will be used in any analysis using genotype data.

Data analysis:
Aim 1. To determine if GCF levels of IL-1β are associated with incident cancer (total, lung, pancreatic or colorectal) or cancer deaths we will use Cox proportional hazards regression to estimate hazard ratios (HR) and 95% confidence intervals (CI) under an additive model or co-dominant model adjusting for race, gender, age, field center, diabetes, smoking and education. The analysis will be repeated stratified by race.
Aim 2. To determine if SNPs in the IL-1β and IL-37 region are associated with incident cancer (total, lung, pancreatic or colorectal) or cancer deaths we will use Cox proportional hazards regression to estimate hazard ratios (HR) and 95% confidence intervals (CI) under an additive model or co-dominant model adjusting for gender, age, field center, diabetes, smoking, education and Ancestry PC’s. The analysis will be stratified by race.

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? __X__ Yes  ____ 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”? _X___ 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/mantrack/maintain/search/dtSearch.html

__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* _1996.01________)  
      ____  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 https://www2.cscc.unc.edu/aric/approved-ancillary-studies

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.