ARIC Manuscript Proposal #4223 (Addendum)

PC Reviewed: 6/13/23 **Status: _____** **Priority: 2**
SC Reviewed: _____ **Status: _____** **Priority: ____**

1.a. Full Title: All-Cause Mortality and Disease-Specific Mortality in Patients with Clonal Hematopoiesis of Indeterminate Potential (CHIP): The Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): All-Cause and Disease-Specific Mortality in Patients with CHIP, an ARIC Study

2. Writing Group:
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I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. SS **[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 data needed for this analysis will be available in 6 months; we plan to submit for publication within 1.5 year.

4. Rationale:

As we age, genetic mutations happen in our cells, causing changes that were not present in our germline DNA, a phenomenon named somatic mosaicism. Like other stem cells, hematopoietic stem cells (HSC) have an increased chance of developing mosaicism. (1) A clone with survival benefits can expand and populate the peripheral blood (i.e., clonal hematopoiesis, CH). (2) The subset of CH with a driver mutation in one of the genes implicated in hematologic malignancies with variant allele frequency (VAF) of at least 2% in the absence of known hematologic malignancy or other clonal disorders is called clonal hematopoiesis of indeterminate potential (CHIP). (3)

Multiple studies confirmed CHIP as an age-related phenomenon that is rare before age 40 and increases to 5%-10% at age 70. (4-6) Multiple studies confirmed an increased odds of coronary artery disease, (6-10) stroke, (6) and heart failure (11, 12) in patients with CHIP. Additionally, CHIP is found to be associated with a 1.4-fold increase in all-cause mortality, which is not entirely because of hematologic malignancies and these patients are at increased risk of cardiovascular mortality as well. (5, 6). However, most of these studies are conducted in middle age people and the prognostic significance of CHIP in older adults is less studied.

With sequencing data becoming more available recently, more people will have CHIP status available. Efforts have been made to elaborate on the prognostic effect of CHIP, and recently the CHIP Risk Score (CHRS) was introduced. (13, 14) The CHRS uses demographic, CBC parameters, and details of the driver mutation and divides people with CHIP into mild, moderate, and high-risk groups.

We will use ARIC visit 5 cohort to define the prognostic significance of CHIP in older adults and study the utility of CHRS in predicting overall and disease-specific survival in individuals with CHIP.

5. Main Hypothesis/Study Questions:

Aim 1: Comparing the mortality rate and disease-specific mortality in individuals with and without CHIP at V5

Hypothesis 1: CHIP is associated with an increased rate of mortality

Hypothesis 2: Causes of death are different in individuals with and without CHIP

Aim 2: Investigating whether CHRS can predict overall and disease-specific survival in individuals with CHIP

Hypothesis 1: Individuals with low-risk CHIP have the same survival as those without CHIP

Hypothesis 2: Individuals with high-risk CHIP have an increased all-cause mortality

Hypothesis 3: Individuals with high-risk CHIP have increased cardiovascular mortality and death from hematologic malignancies

Hypothesis 4: Externally developed CHRS can predict the overall and disease-specific survival of individuals with CHIP in the ARIC cohort

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: ARIC participants with exome sequencing and CBC data available at visit 5 (ARIC-V5, 2011–2013) without hematologic malignancies at baseline will be included.

Exclusions:

- No exome sequencing available for CHIP calling
- Sex-mismatched sequencing samples

Exposure:

- Presence of CHIP at visit 5
- CHRS risk groups

Outcomes:

1. Primary outcome: Overall survival
2. Secondary outcome: Disease-specific survival including cardiovascular-, neurologic-, respiratory-, hematologic-, infectious-, and cancer-survival

Covariates:

Demographic including age, sex, race/ethnicity; clinical conditions including myocardial infarction, stroke, coronary revascularization, history of other atherosclerotic cardiovascular diseases including peripheral vascular disease (PAD), solid organ malignancy, hematologic malignancy, hypertension, diabetes mellitus, dyslipidemia; lifestyle factors including obesity, cigarette smoking, alcohol use, diet, physical activity,

body mass index (BMI); lipid-lowering medication use, antihypertensive medication use, glucose-lowering medication use; and laboratory markers including complete blood count (CBC).

CHIP will be determined using exome sequencing (ES) of blood DNA using the GATK MuTect2 (15) somatic variant caller based on the 74 prespecified driver sequence variations in genes known to promote clonal expansion of hematopoietic stem cells. (6, 7, 16) A conventional variant allele frequency (VAF) of >2% will be used to identify CHIP. and those with VAF >10% will be considered large clones. CHIP calling will be conducted at BROAD institute.

Statistical Analyses:

Continuous variables will be reported using mean (SD) or median (IQR) depending on the normality of the data, while categorical variables will be expressed as count (percentage).

Participants will be categorized based on their CHIP status and for those with CHIP, the CHRS will be calculated using 8 factors, including a) single DNMT3A (present: 0.5 points, absent: 1 point); b) high-risk mutation (absent: 1 point; present: 2.5 points); c) number of mutation (1: 1 point, ≥2: 2 points); d) variant allele fraction (<0.2: 1 point, ≥0.2, 2 points); e) red cell distribution width (<15: 1 point, ≥15: 2.5 points), f) mean corpuscular volume (<100: 1 point; ≥100: 2.5 points); g) cytopenia (absent: 1 point; present: 1.5 points); h) age (<65: 1 point, ≥65: 1.5 points). The high-risk mutation was defined as a mutation in splicing factors (SF3B1, SRSF2, and ZRSR2), AML-like genes (IDH1, IDH2, RUNX1, and FLT3), JAK2, and TP53. The cytopenia was defined as hemoglobin <13 g/dL in males or <12 g/dL in females, platelets <150,000 uL-1, or granulocyte absolute count <1800 uL-1. Individuals with be categorized into low- (CHRS ≤ 9.5), intermediate- (9.5 < CHRS < 12.5), and high- (CHRS ≥ 12.5) risk groups. (13)

Follow-up will be from visit 5 until death or December 31, 2019, except for participants from Jackson center, for whom the last follow-up was December 31, 2017. We will use Fine-Gray competing risk regression to quantify the association between exposures and outcomes. (17) Covariates are age, sex, race, center, diabetes, smoking, coronary artery disease (CAD), heart failure, and history of solid cancer. All statistics will be performed using R (R Foundation, Vienna, Austria, V4.2.2), and statistical significance will be assigned as $P<0.05$.

Sensitivity Analyses:

- NA

<u>Limitations</u>:

- Small sample size: subgroup analyses of disease-specific events may be underpowered.
- There is the potential for residual confounding.

7.a. Will the data be used for non-CVD analysis in this manuscript? Yes

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? Done.
(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

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"? Confirmed

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

Yes

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)? #4100, #3862

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? Yes

11.b. If yes, is the proposal

___ A. primarily the result of an ancillary study (list number:)

_*_ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* (2020.10**)**

*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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