ARIC Manuscript Proposal #3848

PC Reviewed: 5/11/21	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Proteomic age acceleration and mortality in cancer survivors: The Atherosclerosis Risk in Communities Study

b. Abbreviated Title (Length 26 characters): Proteomic age acceleration and mortality in cancer survivors

We have an ancillary proposal (AS2021.06) entitled "Proteomic Aging Clock and Cancer: Atherosclerosis Risk in Community study" was approved in ARIC.

2. Writing Group:

Writing group members: Writing group members: Shuo Wang, Anna Prizment, Elizabeth Platz, Bharat Thyagarajan, Anne Blaes, Jim Pankow, Weihua Guan, Josef Coresh, Corrie Joshu (authorship order TBD and other ARIC researchers are welcome to participate).

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

First author: **Shuo Wang**
Address: 1300 South 2nd Street, Suite 300
Minneapolis, MN 55454

Phone: Fax:
E-mail: wang8310@umn.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).
Name: **Anna Prizment**
Address: 420 Delaware Street SE, MMC 480
Minneapolis, MN 55455

Phone: 612-301-1860 Fax:
E-mail: prizm001@umn.edu

3. Timeline: Analyses will begin within one year after approval.

4. Rationale:

The increased life expectancy in the US coupled with improved cancer survival rates has led to a rapid rise in the number of cancer survivors: there are an estimated 16.9 million cancer survivors as of January 2019, which is projected to rise to 26.1 million by 2040 in the US.[1] However, cancer survivors experience pre-mature mortality from causes other than their cancer(s) and multiple comorbidities.[2, 3] In general, clinicians have noticed that cancer survivors are facing accelerated aging – conceptualized as their biological age is greater than their chronological age – and this manifests as experiencing signs of aging earlier in life than what is expected.[4, 5] It has been shown that accelerated aging in cancer survivors, in first turn, may be caused by cancer treatment.[5] A biological mechanism underlying the accelerated aging involves stress induced by cancer therapies. This stress can induce hallmarks of aging: cellular senescence, telomere attrition, stem cell exhaustion, DNA damage, and epigenetic alterations.[6] In addition to cancer treatment, accelerated aging in cancer survivors may be caused by cancer-related inflammation and unhealthy lifestyle, including persistent shared risk factors for cancer and other chronic diseases.

The evidence for accelerated aging in cancer survivors also comes from the studies comparing life expectancy in cancer survivors to the age-matched general population, with the majority of these data coming from long-term studies on childhood cancer survivors.[7-9] For example, a summary (2009) from the Childhood Cancer Survivor Study (CCSS) compared all-cause mortality rate for 5-year survivors of childhood cancer to the age-adjusted expected all-cause mortality rate for the US general population. They found cancer survivors had a lower survival rate compared to the US general population of the same age: the survival rates after 30+ years from diagnosis were14.22 deaths and 2.07 deaths per 1,000 person-years for cancer survivors and the US general population, respectively.[7] A recent CCSS study (n = 6,148) assessed the risk of late mortality (more than five years after diagnosis) in long-term survivors of childhood acute lymphoblastic leukemia (ALL) as a result of their cancer treatment. With a maximum of 20 years follow-up, they found that the overall late mortality rate was 360% (95% CI: 4.2–5.1) higher than the rate in their age-, sex-, and race-matched US population.[8] Furthermore, Yeh et al. developed a model that estimated the life expectancy of five-year ALL survivors who had been diagnosed with cancer at age 10. The estimated life expectancy for those ALL survivors was 50.6 years, which equals to a loss of 10.4 years compared to the general population.[9] These observations have led to the search for measures of aging, not just in survivors of childhood cancers, but also in survivors of adult cancers, which can be used to screen the aging process and predict the risk of mortality in cancer survivors. Recently, aging clock, which combines a set of molecules capable, has been proposed to be a potential biological age estimator. Several aging clocks have been created, including epigenetic clock and proteomic aging clock.

The most recognized aging clock is the epigenetic clock, a set of DNA methylation-based biomarkers in blood or tissue.[10, 11] To date, only a limited number of studies have measured epigenetic clocks in cancer survivors. For example, a small case-control study measured an epigenetic clock in women with and without ovarian cancer. They reported that cases were estimated to be 1.70 years older than controls.[12] Further, a pooled analysis of seven prospective studies of adult cancer survivors found that overall mortality was 15-30% higher for the highest versus lowest quartile of the epigenetic age acceleration, which was calculated by regressing epigenetic clock on chronological age, independently of major health risk factors.[13]

Recently, a growing body of research supports associations between protein levels and chronological age.[14] A proteomic clock would be attractive because protein biomarkers are easily measurable, they link genotype to phenotype, and can reveal direct information on biological pathways that are involved in many physiological and pathological manifestations of aging.[15, 16] Such proteomic aging clocks may predict the risk of mortality and the development of chronic diseases such as cognitive impairment and frailty.[17-19]

We conducted a preliminary analysis of proteomic age acceleration and overall mortality in cancer survivors using the Atherosclerosis Risk in Communities (ARIC) data. In the preliminary analysis, we built the proteomic aging clock using the 68 proteins in ARIC from the 76 proteins reported in the proteomic aging clock developed by Tanaka et al.,[15] and calculated proteomic age acceleration by regressing proteomic aging clock on chronological age. We found that proteomic age acceleration was associated with overall mortality in cancer survivors after adjusting for age, sex, race, study center, estimated glomerular filtration rate (eGFR), education, BMI, smoking, alcohol, physical activity, aspirin, and diabetes (per one year: HR = 1.16, 95% CI: 1.11 – 1.23). We were interested in eGFR because it was shown to be correlated with SomaScan biomarkers in a polit ARIC study. To better understand the aging process in cancer survivors, we will use the Tanaka clock as well as a new predictive model of aging based on proteomic aging clock and its acceleration specific to cancer that we develop in #MP3739. Acceleration will be calculated by regressing the proteomic aging clock on chronological age. Our aim is to investigate the association between proteomic age acceleration in cancer survivors and all-cause and mortality from causes other than their cancer(s). An understanding of the aging process in cancer survivors would help identify survivors who age faster and need interventions to slow the accelerated aging process.

We have previously proposed to study the association between proteomic age acceleration and risk of cancer in participants without a cancer history (#MP3739) in the ARIC study. In this proposed study, the proteomic aging clock and proteomic age acceleration will be estimated as discussed in #MP3739. The proteins (~5,000 proteins) were assayed using highly sensitive aptamer-based protein profiling (SOMAscan® Platform) in plasma collected at Visits 2, 3, and 5.

We will analyze data from 844 cancer survivors who survived (after their last cancer diagnosed) at least 2 years before blood collection (Visit 2 (1990 - 1992), Visit 3 (1993 - 1995), and Visit 5 (2011-2013)), so that most of those individuals do not undergo active cancer treatment.

5. Main Hypothesis/Study Questions:

The goal of this study is to estimate the association between proteomic age acceleration to the future risk of death, including from other causes in survivors of adult cancers, using the Tanaka clock and a new proteomic clock that we are developing.

Specific Aim 1. Determine the associations of proteomic age acceleration in cancer survivors with the risk of overall mortality and mortality from causes other than their cancer(s).

SA1a. Determine the association between proteomic age acceleration in cancer survivors and the risk of overall mortality.

SA1b. Determine the association between proteomic age acceleration in cancer survivors and the risk of mortality from causes other than their cancer(s).

Hypothesis 1: A higher proteomic age acceleration is associated with an increased risk of mortality in cancer survivors.

Specific Aim 2. Determine if over-time change in proteomic age acceleration is greater among cancer survivors who survived cancers between Visit 2 and at least 2 years before Visit 5 compared to their age-, sex-, and race-matched participants who never had cancer but survived untill visit 5.

Hypothesis 2: Compared to participants who remain cancer-free, participants with no history of cancer at Visit 2 who became diagnosed with cancer will have steeper over-time change in proteomic age acceleration.

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 population

Inclusion criteria: All individuals, who attended ARIC study at Visit 2 (1990 - 1992), Visit 3 (1993 - 1995), or Visit 5 (2011- 2013), had proteins measured by SomaScan and diagnosed with their last cancer at least 2 years before blood collections (Visit 2, Visit 3, or Visit 5) will be eligible for inclusion into the study. These inclusion criteria minimize the likelihood of including survivors who still have active disease in the study.

All individuals without cancer at the time that they attended ARIC study at Visit 2 (1990 - 1992) and Visit 5 (2011- 2013) and had proteins measured by SomaScan will also be eligible for inclusion into the study (Aim 2).

Exposure: Circulating levels of proteins will be extracted from the SomaScan assay (v.4) (SomaLogic company).[20, 21] Using this assay, the ARIC study has measured more than 5000 plasma proteins in frozen plasma samples collected at Visit 3 (1993-1995, N=11,471) and Visit 5 (2011-2013, N=5,193) with excellent within-sample precision for all proteins (the median coefficient of variance: < 7%).[22] Currently, the proteins are being measured in samples collected at Visit 2 (1990-1992, N = 12,769).

We will model proteomic aging clocks at Visit 2, Visit 3, and Visit 5 base on the methods proposed in #MP3739. In brief, first, we will randomly choose a group of 1000 cancer-free people at Visit 5 and used as a training set. Second, using the training set, we will rank proteins based on their standardized ratios (mean intra-individual changes between Visit 2 and Visit 5/standard deviation). The top 1200 proteins (about 25% of 5000 proteins) will be used to create clocks at Visit 2, Visit 3 and Visit 5 by applying a penalized model, such as

elastic net. Third, we will test proteomic aging clocks in the validation set, the rest of cancer-free people at Visit 5, to make sure these proteomic aging clocks are valid. The validity of the clock will be checked by testing correlation between proteomic aging clock with chronological age. A proteomic aging clock will be considered useful if its correlation with chronological age exceeds 0.7 in the validation set. If the correlation does not reach 0.7, we will select more proteins, e.g., the top 2000 proteins with the largest standardized ratios.

Our primary exposure is the proteomic age acceleration. The proteomic age acceleration at each visit will be calculated by regressing each participant's proteomic aging clock on their chronological age at the corresponding visit.

Outcome:
a) Mortality: Mortality case files since inception of ARIC in 1987 were generated and updated annually through December 31, 2019. All participants or their proxies are contacted annually by phone. Deaths were identified through records obtained from hospitals in the ARIC surveillance catchment areas, death certificates, and interviews of next of kin for potential out-of-hospital fatal events or obituaries. Death certificates from state vital statistics offices were obtained on an ongoing basis. Questionnaires were also sent to participants' physicians to confirm out-of-hospital deaths. Dates and underlying causes of death for study participants were verified by death certificate review. Death due to the non-index cancer is defined as a death in which the underlying cause is not attributed to the cancer(s) that a cancer survivor was diagnosed with.

Other covariates of interest: Demographic and clinical variables of interest such as chronological age (at the time of blood collection), sex, race, education, BMI (at the time of blood collection), and date of the cancer diagnosis. Cancer risk factors that are also strongly associate with all-cause mortality (especially major causes of death, cardiovascular disease (CVD), diabetes), such as smoking status, cigarette years of smoking, alcohol intake, and diabetes, will also be extracted at the time of blood collection. Information about eGFR, use of aspirin, use of hormone therapy, history of CVD, will also be extracted at the time of blood collection. We will also extract cancer stage and grade at diagnosis and first of course treatment, where available.

Statistical Analysis

Specific Aim 1 Determine the associations of proteomic age acceleration in cancer survivors with the risk of overall mortality and mortality from causes other than their cancer(s).

SA1a. Determine the association between proteomic age acceleration in cancer survivors and the risk of overall mortality.

SA1b. Determine the association between proteomic age acceleration in cancer survivors and the risk of mortality from causes other than their cancer(s).

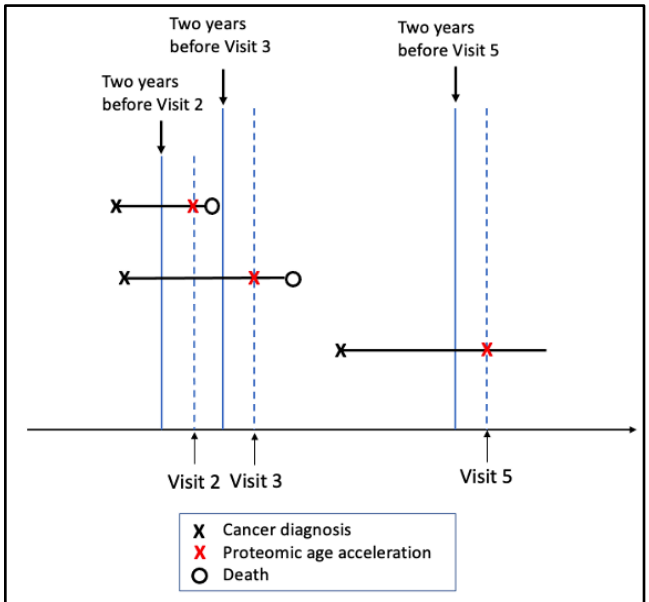

Figure 1. Proteomic age acceleration for cancer survivors

Cox proportional hazards regression will be used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for overall, and mortality from causes other than their cancer(s) in cancer survivors. Proteomic age acceleration will be reported in two ways: continuous and categorized in quartiles. We will model proteomic age acceleration as a time-dependent variable (Figure 1). Person-years will be estimated from the date of blood collection (at Visit 2, Visit 3, or Visit 5, depending on the date of cancer diagnosis) until the death, censor, or the end of follow-up (12/31/2019), whichever occurred first. The analyses will be a priori adjusted for chronological age at the time of blood collection, sex, and race*center. In additional analysis, we will adjust for eGFR, BMI, smoking status, diabetes, history of CVD, use of aspirin, use of hormone therapy, use of metformin, the time between cancer diagnosis and blood collection, cancer stage and grade at diagnosis, and first of course treatment to take into account know factors that influence risk of death or that may explain differences among cancer sites in factors that may influence risk of death. For the analysis of mortality from causes other than their cancer(s) (SA1b), we will treat death from their cancer(s) as a competing risk.

We will also do a sensitivity analysis by only including cancer survivors whose last cancer diagnoses were at least 5 years before their blood collection.

If we have sufficiently power, we will detect if the associations vary by sex and race.

Specific Aim 2. Determine if over-time change in proteomic age acceleration is greater among cancer survivors who survived cancers between Visit 2 and at least 2 years before Visit 5 compared to their age-, sex-, and race-matched participants who never had cancer but survived till visit 5.

Cancer survivors will be included if they were cancer-free at Visit 2 and became diagnosed with cancers at least two years before Visit 5. Using propensity score matching, each cancer survivor will be matched by age, sex, and race with up to 4 randomly selected participants who are cancer-free and survived till Visit 5 (Figure 2). We will evaluate the proteomic age acceleration at Visit 2 and Visit 5 among cancer survivors and participants with no cancer history. We will compute the over-time change in proteomic age acceleration among cancer survivors and their matched cancer-free participants. Linear regression will be used to calculate the risk difference (RD) and 95% CI for the over-time change in proteomic age acceleration. We will adjust for eGFR, BMI, smoking status, diabetes, history of CVD, use of aspirin, use of hormone therapy, and use of metformin to see if this would change the association.

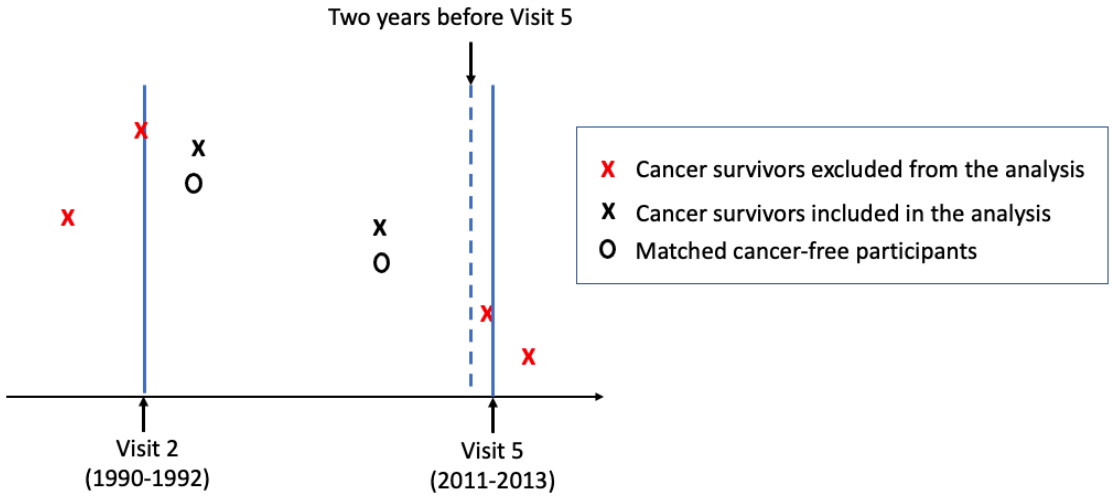

Figure 2. Inclusion and exclusion criteria for comparison of over-time change in proteomic age acceleration between Visit 2 and Visit 5

Power calculations

1) The power calculation for **Specific Aim 1** (mortality up to 2019) is presented in Table 1.

Table 1. Minimal detectable hazard ratio (power=80% two-sided α= 0.05) for the association of proteomic age acceleration with mortality (overall and non-cancer) in cancer survivors who survived at least 2 years before blood collection

Outcome	No. of cancer survivors[a]	No. of incident outcomes	Minimal detectable HR
All-cause mortality	844	344	1.35
Mortality from causes other than their cancer(s)	844	320	1.37

[a]Cancer survivors survived at least 2 years before blood collection

2) The power calculation for **Specific Aim 2** is presented in Table 2.

Table 2. Power calculation in ARIC participants (80% power, alpha = 0.05, two-sided) for comparison of over-time change in proteomic age acceleration between Visit 2 and Visit 5 in cancer survivors and their matched cancer-free participants

Outcome	Number of cancer survivors[a]	Number of people without cancer	Minimal detectable RD
Over-time change in proteomic age acceleration	760	3,040	0.11

[a] Cancer survivors who were cancer-free at Visit 2 and became diagnosed with cancers at least 2 years before Visit 5.

7.a. Will the data be used for non-ARIC analysis or by a for-profit organization in this manuscript? ____ Yes _X__ No

b. If Yes, is the author aware that the current derived consent file ICTDER05 must be used to exclude persons with a value RES_OTH and/or RES_DNA = "ARIC only" and/or "Not for Profit" ? ____ Yes ____ No
(The 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 current derived consent file ICTDER05 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/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)?

#MP3445 The Association of Systemic inflammation with Mortality Due to Non-Index Cancer in Older Adult Cancer Survivors.

#MP3739 Proteomic age acceleration and cancer incidence: The Atherosclerosis Risk in Communities Study

#MP3515 A comparison of the inflammatory proteome in cancer survivors and individuals with no cancer history.

#MP3482 Plasma Proteins and All-Cause Mortality in Cancer Survivors in ARIC

#MP3057 Repeatability and Longitudinal Variability of the Plasma Proteome.

#MP3617 Association between functional MICA polymorphisms, soluble MICA levels, colorectal cancer incidence and mortality: Results from the Atherosclerosis Risk in Communities study.

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* _____)

AS1995.04 Cancer Study
AS2011.07 Enhancing ARIC Infrastructure to Yield a New Cancer Epidemiology Cohort
AS2017.27 Proteomic longitudinal ARIC study: SOMAscan of multiple visits

___ 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 https://sites.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.

References

1. Institute NC. statistics, Graphs, and Definitions. Accessed Jan 4, 2021. https://cancercontrol.cancer.gov/ocs/statistics#stats
2. Cupit-Link MC, Kirkland JL, Ness KK, et al. Biology of premature ageing in survivors of cancer. *ESMO Open*. 2017;2(5):e000250. doi:10.1136/esmoopen-2017-000250
3. Suh E, Stratton KL, Leisenring WM, et al. Late mortality and chronic health conditions in long-term survivors of early-adolescent and young adult cancers: a retrospective cohort analysis from the Childhood Cancer Survivor Study. *Lancet Oncol*. 03 2020;21(3):421-435. doi:10.1016/S1470-2045(19)30800-9
4. Sebastiani P, Thyagarajan B, Sun F, et al. Biomarker signatures of aging. *Aging Cell*. Apr 2017;16(2):329-338. doi:10.1111/acel.12557
5. Hurria A, Jones L, Muss HB. Cancer Treatment as an Accelerated Aging Process: Assessment, Biomarkers, and Interventions. *American Society of Clinical Oncology educational book American Society of Clinical Oncology Annual Meeting*. 2016;35:e516-22. doi:10.1200/edbk_156160
6. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell*. Jun 2013;153(6):1194-217. doi:10.1016/j.cell.2013.05.039

7. Armstrong GT, Liu Q, Yasui Y, et al. Late mortality among 5-year survivors of childhood cancer: a summary from the Childhood Cancer Survivor Study. *J Clin Oncol*. May 2009;27(14):2328-38. doi:10.1200/JCO.2008.21.1425
8. Dixon SB, Chen Y, Yasui Y, et al. Reduced Morbidity and Mortality in Survivors of Childhood Acute Lymphoblastic Leukemia: A Report From the Childhood Cancer Survivor Study. *J Clin Oncol*. Oct 2020;38(29):3418-3429. doi:10.1200/JCO.20.00493
9. Yeh JM, Nekhlyudov L, Goldie SJ, Mertens AC, Diller L. A model-based estimate of cumulative excess mortality in survivors of childhood cancer. *Ann Intern Med*. Apr 2010;152(7):409-17, W131-8. doi:10.7326/0003-4819-152-7-201004060-00005
10. Hannum G, Guinney J, Zhao L, et al. Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol Cell*. Jan 24 2013;49(2):359-367. doi:10.1016/j.molcel.2012.10.016
11. Horvath S. DNA methylation age of human tissues and cell types. *Genome Biol*. 2013;14(10):R115. doi:10.1186/gb-2013-14-10-r115
12. Galkin F, Mamoshina P, Kochetov K, Sidorenko D, Zhavoronkov A. DeepMAge: A Methylation Aging Clock Developed with Deep Learning. *Aging Dis*. 2020;
13. Dugué PA, Bassett JK, Joo JE, et al. DNA methylation-based biological aging and cancer risk and survival: Pooled analysis of seven prospective studies. *Int J Cancer*. Apr 15 2018;142(8):1611-1619. doi:10.1002/ijc.31189
14. Johnson AA, Shokhirev MN, Wyss-Coray T, Lehallier B. Systematic review and analysis of human proteomics aging studies unveils a novel proteomic aging clock and identifies key processes that change with age. *Ageing research reviews*. Jul 2020;60:101070. doi:10.1016/j.arr.2020.101070
15. Tanaka T, Biancotto A, Moaddel R, et al. Plasma proteomic signature of age in healthy humans. *Aging Cell*. Oct 2018;17(5):e12799. doi:10.1111/acel.12799
16. Nussinov R, Tsai CJ, Jang H. Protein ensembles link genotype to phenotype. *PLoS Comput Biol*. 06 2019;15(6):e1006648. doi:10.1371/journal.pcbi.1006648
17. Lehallier B, Gate D, Schaum N, et al. Undulating changes in human plasma proteome profiles across the lifespan. *Nat Med*. Dec 2019;25(12):1843-1850. doi:10.1038/s41591-019-0673-2
18. Tanaka T, Basisty N, Fantoni G, et al. Plasma proteomic biomarker signature of age predicts health and life span. *Elife*. 11 2020;9doi:10.7554/eLife.61073
19. Sathyan S, Ayers E, Gao T, et al. Plasma proteomic profile of age, health span, and all-cause mortality in older adults. *Aging Cell*. Nov 2020;19(11):e13250. doi:10.1111/acel.13250
20. Gold L, Ayers D, Bertino J, et al. Aptamer-based multiplexed proteomic technology for biomarker discovery. *PLoS One*. Dec 7 2010;5(12):e15004. doi:10.1371/journal.pone.0015004
21. Candia J, Cheung F, Kotliarov Y, et al. Assessment of Variability in the SOMAscan Assay. *Sci Rep*. 10 2017;7(1):14248. doi:10.1038/s41598-017-14755-5
22. Tin A, Yu B, Ma J, et al. Reproducibility and Variability of Protein Analytes Measured Using a Multiplexed Modified Aptamer Assay. *The journal of applied laboratory medicine*. Jul 2019;4(1):30-39. doi:10.1373/jalm.2018.027086