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Associations of Seven Measures of Biological Age Acceleration with Frailty and All-Cause Mortality Among Adult Survivors of Childhood Cancer in the St. Jude Lifetime Cohort

Jennifer L. Guida¹, Geehong Hyun², Daniel W. Belsky³, Gregory T. Armstrong², Matthew J. Ehrhardt², Melissa M. Hudson², Paige A. Green¹, Leslie L. Robison², Brennan P. Streck¹, Emily S. Tonorezos⁴, Yutaka Yasui², Carmen L. Wilson², Zhaoming Wang^{2,*}, Kirsten K. Ness^{2,*}

¹Division of Cancer Control and Populations Sciences, National Cancer Institute, 9609 Medical Center Drive, Rockville, MD 20850, USA 12

²Department of Epidemiology and Cancer Control, St. Jude Children's Research Hospital, 262 Danny Thomas Pl, Memphis, TN 38105, USA 15

³Department of Epidemiology and Butler Columbia Aging Center, Columbia University Mailman School of Public Health, 722 W 168th Street, New York, NY 10032, USA 18

⁴Office of Cancer Survivorship, National Cancer Institute, 9609 Medical Center Drive, Rockville, MD 20850, USA 21

Abstract

Survivors of childhood cancer may experience accelerated biological aging, resulting in premature frailty and death. We used seven measures of biological age in the St. Jude Lifetime (SJLIFE) Cohort to compare biological age acceleration among SJLIFE and NHANES III controls, explore trajectories of biological age by cancer treatment and type, and test associations of biological age acceleration with frailty and death (mean follow-up of 26.5 years) among survivors. Cancer survivors aged 5% faster per year and measured, on average, 0.6–6.44 years biologically older compared to controls and 5–16 years biologically older compared to age-matched individuals at the population level. Survivors treated with hematopoietic cell transplant and vinca alkaloid

*Co-corresponding Authors: Kirsten K. Ness, PT, PhD, FAPTA, Department of Epidemiology and Cancer Control, St. Jude Children's Research Hospital, 262 Danny Thomas Place, MS-735, Memphis, TN 38105, kiri.ness@stjude.org, Zhaoming Wang, PhD, Department of Epidemiology and Cancer Control, St. Jude Children's Research Hospital, 262 Danny Thomas Place, MS-735, Memphis, TN 38105, zhaoming.wang@stjude.org.

Author Contributions

All authors, JLG, GH, DWB, GTA, MJE, MMH, PAG, LLR, BPS, EST, YY, CLW, ZW, and KKN, contributed to the manuscript's study design, writing, reviewing, and editing. MMH, GTA, LLR, KKN, ZW, GH, CLW, and YY performed material preparation and data collection. ZW and GH provided data curation, and GH conducted the statistical analysis. JLG provided project administration and wrote the original draft; KKN and PAG supervised the project. All authors read and approved the final manuscript.

Competing Interests

We declare no competing interests. This work is solely the authors' responsibility and does not necessarily represent the official views of the National Cancer Institute or the National Institutes Health.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Code Availability

The code supporting this study's findings is publicly available online at <https://zenodo.org/records/10035257>.

chemotherapy evidenced the fastest trajectories of biological aging. Biologically, older and faster-aging survivors consistently and robustly had a higher risk of frailty and died earlier than those with slower biological aging, suggesting a potential opportunity to intervene on excess aging.

Keywords

childhood cancer; cancer survivor; biological age; accelerated aging; epigenetics; epigenetic clock; DNA methylation; frailty; mortality; biological sciences/cancer

Childhood cancer survival has improved dramatically over the past five decades, with over 85% of patients expected to live at least ten years after diagnosis¹. However, these achievements in lifespan do not equate to advances in healthspan. Growing evidence suggests that adult survivors of childhood cancer experience accelerated biological aging^{2,3}, which may result in the early onset of frailty^{4,5} and premature death^{6,7}. The Reverse Geroscience Hypothesis posits that chronic diseases, such as cancer and their treatments, cause cellular damage that mimics or targets the hallmarks of aging (e.g., epigenetic alterations, DNA damage, cellular senescence)⁸. Over time, the accumulation of cellular damage may lead to accelerated biological aging⁹, premature onset of aging phenotypes (i.e., frailty), and ultimately, early death^{3,10}. Consistent with the Reverse Geroscience Hypothesis, childhood cancer survivors show laboratory signs of premature biological aging, including telomere attrition, cellular senescence, and inflammation^{11–19}, and specific cancer treatments have been linked to aging phenotypes, such as frailty⁵. Predictive biomarkers of accelerated aging phenotypes are needed to identify survivors at risk of premature aging and those who might benefit from preventive interventions^{3,20}.

A new generation of aging biomarkers has the potential to meet this need. These biomarkers are based on algorithms that integrate information from multiple clinical or molecular measurements to estimate an individual's biological age or pace of aging. Initial studies suggest that at least some of these measures indicate advanced or accelerated biological aging in survivors of childhood cancer^{13,16}. However, these measures are rarely estimated in the same population, and consensus on the best biological measure of aging has not been reached, making comparisons across measures difficult. We used a large cohort of childhood cancer survivors and matched controls to evaluate a panel of the best-evidenced and most-studied of these novel aging biomarkers to test the Reverse Geroscience Hypothesis. We included seven measures of biological aging in our analysis: two physiology-based algorithms (the Klemera-Doubal Biological Age (KDM-BA) and PhenoAge), four DNA methylation (DNAm) clocks (the Horvath, Hannum, PhenoAge, and GrimAge clocks), and DunedinPACE, a single-time-point DNAm blood-test of the speed of biological aging (Supplementary Table 1). We compared adult survivors of childhood cancer in the St. Jude Lifetime (SJLIFE) cohort with two control groups: (1) community controls from the SJLIFE cohort (herein referred to as community controls) and (2) participants in the third U.S. National Health and Nutrition Examination Survey (NHANES III) to test if cancer survivors were biologically older and aging more rapidly, as compared to non-cancer controls. Among the SJLIFE survivors, we tested if specific cancer types and treatments influenced the trajectory of biological aging over a mean follow-up period of 26.5 years using physiology-

based measures. We also assessed whether participants with older biological age and a faster pace of aging were at increased risk of frailty and mortality. We hypothesize that survivors will be biologically older and aging faster as compared to SJLIFE and NHANES III controls, specific cancer types and treatments will accelerate biological age cross-sectionally and longitudinally, and survivors who are biologically older and aging faster will have a higher risk of frailty and die earlier than those with slower biological aging.

Results

Characteristics of the Study Population

A total of 4,117 adult survivors of childhood cancer, 606 community controls, and 16,421 NHANES III participants were included in analyses of physiology-based biological age, and 2,095 SJLIFE survivors and 282 community controls were included in DNAm analyses (Table 1). The mean chronological age was 35, 34, and 42 years among survivors, community controls, and NHANES III controls, respectively ($p < 0.001$). Survivors were statistically different from the SJLIFE community and NHANES III controls, with higher proportions of men and obesity ($p < 0.001$) and higher mean depression scores ($p < 0.001$) observed in SJLIFE survivors. About half of the survivors participated in 150 minutes of moderate-vigorous physical activity (52.7% versus 61.8% and 62.6% of community and NHANES controls, respectively, $p < 0.001$) and risky drinking (47.1% versus 58.8% and 27.2% of community and NHANES III controls, respectively, $p < 0.001$). 17.8% of survivors reported current smoking compared to 12.3% and 29.0% of community and NHANES III controls, respectively ($p < 0.001$).

The median (standard deviation; SD) age at diagnosis and time since diagnosis among survivors was 8.6 years ($SD = 5.7$ years) and 26.5 years ($SD = 10.4$ years), respectively (Supplementary Table 2). Primary diagnoses comprised leukemia (34.3%), central nervous system (CNS) tumors (13.5%), Hodgkin lymphoma (HL) (11.9%), non-Hodgkin lymphoma (NHL) (6.9%), bone tumors (6.9%), Wilms tumor (5.9%), neuroblastoma (4.3%), soft tissue sarcoma (3.0%), and other solid tumors (13.5%). 29.6% of survivors were exposed to cranial radiation, 17.8% chest radiation, 18.3% abdominal or pelvic radiation, 64.8% vinca alkaloids, 58.8% cyclophosphamide, 57.4% doxorubicin, 43.8% methotrexate, and 34.1% 6-mercaptopurine, and 8% hematopoietic cell transplant (HCT).

Biological Age Acceleration in Survivors and Controls

Age acceleration of KDM-BA, PhenoAge, the DNAm clocks, and original scale values of DunedinPACE were correlated, although patterns varied (Supplementary Table 3). Fig. 1 and Table 2 show the difference in biological age acceleration between survivors and controls (standardized estimates presented in Supplementary Table 4). Survivors were aging at a pace of 5% faster per year than community controls (DunedinPACE $\beta = 0.05$, 95% CI: 0.04–0.06). At a mean chronological age of 35 years, cancer survivors measured, on average, 0.6–1.88 years and 2.22–6.49 years biologically older over their lifetime compared to community controls, as measured by residuals and difference scores, respectively. Moreover, survivors measured 5.2–15.9 years biologically older than expected for someone of their chronological age at the population level. Compared to the NHANES III controls, survivors

were, on average, 0.75–4.94 years biologically older ($p < 0.05$) despite NHANES III controls being chronologically seven years older.

Trajectories of Biological Age by Cancer Site and Treatment

A subset of survivors ($n=1,042$) had physiology-based data available at three or more visits. For PhenoAge, survivors of lymphoma, CNS tumors, and Wilms tumor showed the fastest trajectories of biological aging ($\beta=1.08$ – 1.12 , Supplementary Table 5). In contrast, KDM-BA showed that rates of aging across cancer types were not statistically significant. Cross-sectionally, survivors treated with HSCT, and total body irradiation showed consistent signs of biological aging across measures, with survivors experiencing 1.48–5.17 years and 0.37–9.47 years of advanced biological age for HSCT and total body irradiation, respectively (Supplementary Table 6). Survivors exposed to HCT (overall least square mean difference [OLSMD]=2.41, $\beta=0.47$, 95% CI: 0.03, 0.91) and vinca alkaloid chemotherapy (OLSMD=-0.44, $\beta=0.15$, 95% CI: 0.004, 0.30) had faster rates of biological aging, as measured by KDM-BA and PhenoAge, respectively, than survivors who did not receive the respective treatments (Supplementary Table 7); all other treatments did not show statistically significant differences. 148 Associations of Biological Age Acceleration and Frailty Among survivors, 36.1% were robust ($n=1,468$), 56.8% were pre-frail ($n=2,307$), and 7.1% were frail ($n=287$). After adjustment for age at diagnosis, race/ethnicity, sex, treatment type, BMI, depression, smoking, alcohol use, and physical activity (model 3), survivors with the highest biological age acceleration (tertile 3) were at increased risk of frailty (range of odds ratios: 1.08–3.13, $p < 0.05$), compared to those with the least age acceleration (tertile 1; Table 3, Supplementary Table 8). Horvath's clock-frailty association was calculated with difference scores, and Hannum's clock-frailty association was calculated with residual and difference scores, which were all in the expected direction but did not reach statistical significance.

Associations of Biological Age Acceleration and Morality

The follow-up period included 2,044–3,658 survivors (numbers differ by biological age measure) over 26.5 years, during which 4.1% of participants died ($n=167$). Across all measures of biological age and nested models (Fig. 2, Table 3, Supplementary Table 9), biological age acceleration was consistently and robustly associated with all-cause mortality. For instance, after adjustment for model 3 covariates, survivors with the highest biological age acceleration (tertile 3) were at increased risk of all-cause mortality (range of hazard ratios: 1.77–14.54, $p < 0.05$; Table 3) compared to those with the least age acceleration (tertile 1). Associations were also tested between tertiles of biological age acceleration and cause-specific mortality due to recurrence and SMNs. Comparing those with the highest biological age acceleration to the lowest and using the residualized approach, Horvath's clock (HR=3.52, 95% CI: 1.04–11.90) and DNAmPhenoAge (HR=7.27, 95% CI: 1.53–34.46) were significantly associated with mortality due to SMNs. Hannum's clock (HR=4.68, 95% CI: 1.58, 13.84), DNAmPhenoAge (HR= 4.80, 95% CI: 1.63,14.12) and GrimAge (HR=2.93, 95% CI: 1.06, 8.13) were significantly associated with SMNs when the difference score approach was used (Supplementary Table 10); other measures were in the expected directions but did not reach statistical significance.

Discussion

In this large, retrospective cohort of adult survivors of childhood cancer, we found consistent evidence of biological age acceleration in comparison to community and NHANES III controls. Survivors measured 0.6–6.44 years biologically older compared to community and NHANES III controls and aged 5–16 years older than expected for individuals of the same chronological age at the population level. We also showed survivors aged 5% faster per year than SJLIFE controls, as measured by DunedinPACE. Survivors who were biologically older (measured by KDM-BA, PhenoAge, Hannum’s clock, Horvath’s clock, DNAmPhenoAge, GrimAge) and aging faster (DunedinPACE) had increased risks of frailty and premature mortality over 26.5 years of follow-up. Together, the strength of the associations and the consistency of the findings across seven measures of biological age suggest that epigenetic and physiologic composite measures of biological age may serve as predictive biomarkers of accelerated aging phenotypes (i.e., frailty, accelerated all-cause mortality, and selectively, SMN mortality) in adult survivors of childhood cancer. In addition, the longitudinal collection of physiology-based biological aging measures and the inclusion of the DunedinPACE measure, a measure of the speed of biological aging, allowed us to establish that adult survivors of childhood cancer are aging faster and that specific cancer types and treatments are associated with accelerated aging trajectories, potentially identifying critical opportunities to intervene to prevent future excess aging.

Our findings expand on those from a prior study that reported older DNAmPhenoAge in cancer survivors compared to community controls¹⁶ and illustrates that across a broad array of DNAm and physiology-based measures of biological aging, survivors of childhood cancer appear to be biologically older and aging more rapidly than adults with no cancer history. Moreover, our findings link accelerated biological aging with frailty and shorter lifespan. Together with a growing number of studies documenting altered hallmarks of aging^{12–19} and accelerated aging phenotypes in cancer survivors^{4,5,21,22}, our findings advance the hypothesis that survivors experience shorter healthspan and lifespan due to a combination of disease and its treatment accelerating biological aging processes.

Accelerated biological aging trajectories were most pronounced for HL, NHL, Wilms tumor, and CNS tumors, which is likely due to the intensive treatment regimens utilized for cure. Previous studies report that survivors of HL and CNS tumors are at the highest risk for frailty compared to survivors of other cancers^{5,23}. Wilms tumor and NHL historically used intensive treatment regimens, and these findings may reflect cohort effects, as treatments and regimens have changed in recent years. Another explanation is that the physiology-based measures include blood chemistries, such as blood urea nitrogen and creatinine, which are highly affected by nephrectomy, a common surgery for Wilms Tumor. Therefore, these measures may be more sensitive to the aging caused by Wilms tumor-associated treatment.

Our study adds to the literature by demonstrating that exposure to vinca alkaloid chemotherapy and HCT leads to faster biological aging trajectories using physiology-based metrics. Microtubule-destabilizing agents, including vinca alkaloid chemotherapeutic agents, have been associated with cardiotoxicity^{24,25} and epigenetic alterations, a hallmark of aging. HCT is associated with accelerated aging phenotypes, including high rates of frailty, severe/

life-threatening conditions, and functional limitations^{21,24} and has been linked to the aging hallmarks of telomere attrition and stem cell exhaustion²⁵. Future work is needed to replicate our findings and to further understand whether dose modification or reduction of certain intensive therapies would reduce the onset of aging phenotypes.

Despite consistent findings across the seven measures, Hannum's clock and Horvath's clock (selectively) did not reach statistical significance for the biological age acceleration-frailty association, and the two physiology-based measures showed differing results for the trajectory analysis by cancer treatment and type. This suggests that some biological age measures may be less sensitive for detecting cancer and treatment-related aging. Further, certain measures may contain components that are biological sequelae of specific cancers or their treatments. Because the mechanisms of action that drive changes in the DNAm clocks and other composite biomarkers are not well understood, it remains unclear whether differential performance among the aging measures primarily reflects sensitivity to cancer/treatment-related aging or confounding.

At present, it remains unclear which biomarkers of aging are most appropriate for identifying cancer survivors at risk of accelerated aging. Comprehensive approaches, including those employed in our study, are needed to interrogate consistent signs of aging across multiple biomarkers in the same population to test the Reverse Geroscience Hypothesis. Improving risk prediction by developing biological aging algorithms specific to cancer survivors may be possible. For example, in addition to frailty and reduced lifespan, adult survivors of childhood cancer also experience markedly higher arterial stiffness²⁶ and cognitive decline²². Augmenting biological age algorithms to capture this information may help improve risk prediction, as unmeasured system damage may underestimate true biological age acceleration estimates. Additionally, studies are needed to test the effectiveness and optimal timing of preventive interventions delivered to biologically older/faster-aging cancer survivors.

The strengths of this study include its incorporation of multiple measures of biological aging in a large cohort of adult survivors of childhood cancer with frailty and all-cause mortality outcomes. However, our study is not without limitations. At the time of this analysis, DNAm data were only available at one time point, and we could not assess trajectories of biological aging using the DNAm measures. In the trajectory analysis of physiology-based biological age by treatment type, other cancer survivors treated with anticancer therapies were used as the control group, which may have reduced our ability to detect differences. Second, the SJLIFE survivors were all treated at St. Jude Children's Research Hospital (SJCRH) and may be different than survivors treated at other institutions with differing geographic catchment areas. We used NHANES III controls to partially address this limitation, although only data on physiology-based biological aging measures were available for this group. The smaller sample size for racial/ethnic minority groups, driven by the composition of the study population, limited our ability to conduct subgroup analyses by race/ethnicity. Future studies should determine if there are differences in biological aging and risk of frailty and mortality among survivors from historically underserved and underrepresented groups. CRP and cytomegalovirus were not included in the physiology-based biological aging measures; however, multiple organ systems are still represented in the calculations

to reflect multisystem aging. Lastly, survivor bias may have underestimated the findings, as those with advanced-stage cancer and/or treated with more intensive therapies may have aged most rapidly and died before study enrollment.

In summary, we demonstrated that adult survivors of childhood cancer experience accelerated biological aging compared to cancer-free controls and that among survivors, biological age acceleration was associated with frailty and premature mortality. Moreover, these associations were consistent across seven measures of biological age, suggesting that these markers may be used to identify cancer survivors at risk for accelerated aging.

Methods

The SJLIFE protocol, biospecimen banking, and genomic study were approved by the St. Jude Institutional Review Board and participants provided written informed consent. Participants were compensated \$150 US dollars per day when they completed their clinical assessment.

Study Participants and Setting

SJLIFE is a large, retrospective, observational cohort study of five-year or longer survivors of childhood cancer and community controls^{27,28}. The study was initiated in 2007 with prospective follow-up of survivors and ongoing periodic assessment of medical outcomes and biospecimen collection. The participants in the current study were followed up through December 2018. All SJLIFE participants completed medical and laboratory assessments of their health conditions and a multi-item survey on demographics and health behaviors. Eligible SJLIFE participants were 18+ years of age, diagnosed with a primary malignancy at least five years prior, and were treated at SJCRH for cancer (mean age at diagnosis=8.62 years \pm 5.67 years; mean age at evaluation= 35.08 \pm 10.17 years). Community controls were selected from friends and non-first-degree relatives of former and current SJCRH patients and were frequency-matched on 5-year age blocks within each sex. Because the design is observational and not experimental, participants were not randomized or blinded to a condition. Sex was considered in the study's design and was obtained by medical record abstraction.

A total of 5338 participants from SJLIFE received a campus visit. Participants were included if they completed an on-campus visit and had physiology-based biomarker data (n=4,117 cancer survivors; and n=606 community controls) and DNAm data (n=2,095 survivors and n=282 controls) (Fig. 3). A subset of survivors had three or more campus visits and were included in biological age trajectory analyses (n=1,042). The average follow-up of survivors was 26.5 years \pm 10.37 years from cancer diagnosis; controls were not followed prospectively. Additionally, because several biological age measures were trained in NHANES samples^{29,30} and SJLIFE survivors represent diverse geographic locations, we compared survivors to NHANES III participants (1988–1994)³¹ who were 18 years and older, self-reported no history of cancer and had physiology-based biological age data available (n=16,421). NHANES III epigenetic data were not publicly available at the time of writing and, therefore, were not included.

Measures

Physiology-Based Measures of Biological Age—Supplementary Table 1

summarizes the differences among measures of biological age. The Klemmera-Doubal Method Biological Age (KDM-BA) algorithm³² measures biological age as the age at which a person's physiology would be normal in a reference population. We implemented KDM-BA following the approach of Levine and colleagues²⁹, including eight of the original ten biomarkers in their algorithm (albumin, alkaline phosphatase, blood urea nitrogen, creatinine, forced expiratory volume in one second (FEV1), glycated hemoglobin (HbA1C), systolic blood pressure, and total cholesterol; C-reactive protein and cytomegalovirus optical density were not available in the SJLIFE cohort). 316 The Phenotypic Age (PhenoAge) algorithm^{30,33} measures the biological age at which a person's predicted mortality risk would be approximately normal in a reference population. We implemented PhenoAge using seven of the original nine biomarkers (albumin, alkaline phosphatase, creatinine, lymphocyte percent, mean cell volume, and red cell distribution width; we substituted HbA1C for glucose; C-reactive protein and white blood cell count were not available in SJLIFE).

DNAm Measures of Biological Age—Epigenetic age and epigenetic age acceleration was calculated for SJLIFE cancer survivors and community controls, (n = 2,095 survivors and n = 282 controls) whose DNA extraction was completed with the same protocol in the Computational Biology Genomic Laboratory at St. Jude Children's Research Hospital. Genome-wide methylation data were generated using the Infinium Methylation EPIC BeadChip array (Illumina, San Diego, CA, USA) on the whole blood-derived DNA.³⁴ Genomic DNA (250 ng) was extracted from blood samples according to standard procedures as described in the previous study.¹⁶ The extracted DNA bisulfite was treated using the Zymo EZ DNA Methylation Kit (ZYMO RESEARCH, Irvine, CA) with the following thermos-cycling conditions: 16 cycles: 95°C for 30 sec, 50°C for 1 hour. After bisulfite treatment, DNA samples were desulphonated, column purified, then eluted using 12 ul of elution buffer (Zymo Research). After elution, the entire bisulfite-treated DNA was processed using the Illumina Infinium Methylation Assay including hybridization to HumanMethylation850K EPIC BeadChips, single base extension assay, staining and scanning using the Illumina iScan system according to the manufacturer's protocol.

The raw intensity data (IDAT files) were analyzed using Illumina Genome Studio software to compute the β -values (range = 0–1), to describe the methylation level of a specific CpG site. Quality control was conducted to identify and remove samples and probes with low quality. Probes were excluded if detection rates were <95% with a detection $P < 0.01$ across all samples (or with SNPs at CpG sites), or if samples had low call rates (<95% probes with a detection $P < 0.01$). Cross-reactive and non-specific probes were also excluded from downstream analyses.

DNAm Clocks.—We measured biological age from DNAm data using four DNAm clock algorithms: Horvath's clock³⁵, Hannum's clock³⁶, DNAmPhenoAge³³, and GrimAge³⁷. The Horvath and Hannum clocks were developed to predict chronological age from DNAm data. These clocks are referred to as "First Generation" and are defined as the age at which a person's methylome would be normal in a reference population. The DNAmPhenoAge

and GrimAge clocks were developed to predict mortality risk from DNAm data. The DNAmPhenoAge clock was developed by modeling the physiology based PhenoAge from DNAm data in the InCHIANTI cohort³³. The GrimAge clock was first developed using DNAm predictors of a panel of blood proteins and smoking history and then modeling mortality risk from the DNAm predictors in the Framingham Heart Study Offspring cohort³⁷. DNAmPhenoAge and GrimAge are referred to as “Second Generation” clocks, and they measure biological age as the age at which a person’s DNAm-predicted risk of mortality would be normal in a reference population.

DunedinPACE. DunedinPACE is a DNAm measure of the rate of decline in system integrity³⁸. DunedinPACE was developed by modeling a measure of the pace of aging, a composite of the rates of change in 19 biomarkers of organ system integrity measured over two decades³⁹, from DNAm data in the Dunedin Study birth cohort⁴⁰. Thus, DunedinPACE can be interpreted as whether an individual is biologically aging faster or slower (i.e., the speed of biological aging), whereas the other measures aim to assess how biologically old or young an individual is.

Cancer Type and Treatment Exposure—SJLIFE includes individuals diagnosed with any childhood cancer histology. Detailed information about cancer and cancer treatment exposures was abstracted by trained research staff using a structured protocol, as previously described^{27,28}.

All-Cause and Cause-Specific Mortality—Vital status was assessed via linkage to the National Death Index to obtain all-cause, recurrence-, and subsequent malignant neoplasm (SMN)-specific mortality.

Frailty—Frailty was assessed at the first campus visit and defined by three or more of low lean muscle mass (assessed by DXA scan), self-reported exhaustion (self-reported using the SF-36 vitality subscale), muscle weakness (defined by handgrip strength), slow walking speed (assessed by a 15-foot walk test), and low energy expenditure (estimated using a modified form of the NHANES physical activity questionnaire to calculate calories expended per week) using a modified version of the Fried Frailty Index⁴¹. Except for exhaustion, all thresholds for abnormal were sex-specific (Supplementary Table 11). Frailty components were summed to create a score and classified as 0=robust, 1–2=pre-frail, and 3–5=frail.

Psychosocial and Behavioral Characteristics—Depression (yes/no), smoking status (never/current/former), physical activity (> 150 minutes vs. <150 minutes per week of moderate-intensity physical activity)⁴², and alcohol use (none/moderate vs. risky, defined as ≥ 5 drinks for males or ≥ 4 drinks for females on at least 1 day in the past) were assessed via questionnaire and chosen based on their established associations with biological age in the general population^{30,43}.

Statistical Analysis

The normality assumption was checked for analyses of descriptive statistics. One-way analysis of variance or t-tests were conducted when the normality was not violated.

Non-parametric methods (Wilcoxon rank sum test or Kruskal-Wallis test) were used when the normality violated. Outliers were detected and excluded from linear regression models (<2%) to meet the normality assumptions. The proportional hazards assumptions for mortality analyses by Cox regression models were assessed with Kolmogorov-type supremum tests and stratified Cox regression models were used when the proportional assumption was violated. No statistical methods were used to pre-determine sample sizes, but our sample sizes are similar to those reported in previous publications.¹⁶ Generalized estimating equations were used to estimate trajectories of physiology-based biological age by cancer type and treatment history among survivors with three or more waves of data, adjusting for chronological age. Trajectories of biological age by cancer type were modeled separately, while trajectories by cancer treatment were modeled together to account for other treatment exposures. Biological age acceleration was estimated using two methods: 1) by regressing biological age measures on chronological age and computing residual values, and 2) by calculating difference scores for each biological age measure by subtracting chronological age from the computed clock ages. For DunedinPACE, the original scores were used because this measure is an estimate of the pace of aging, not an age.⁴⁴ Linear regression was used to test cross-sectional differences in biological age acceleration between survivors and controls. Multinomial logistic regression was used to test cross-sectional associations between tertiles of biological age acceleration (reference group=tertile 1) and frailty (reference group=robust).

For mortality outcomes, follow-up started five years from the time of diagnosis and ended at the date of death or censoring (September 2009-December 2018). Kaplan-Meier survival curves were used to illustrate unadjusted all-cause mortality outcomes. Cox regression models were used to test associations of biological age acceleration tertiles with all-cause mortality. Cause-specific mortality was assessed using Fine and Gray models using the proportional sub-distribution hazard function. The proportional hazards assumption was tested, and the hazard ratios (HR) were estimated using stratified Cox regression when the hazard assumption was violated. We conducted three sets of nested models for frailty and mortality analyses for each biological age parameter. The first model covariates included age at diagnosis, race/ethnicity, and self-reported sex; the second model additionally adjusted for treatment type (chemotherapy, radiotherapy, surgery); and the third model included model 1+2 covariates and BMI, depression, smoking, alcohol use, and physical activity. Sex-specific analyses were not conducted because there was no specific, a priori scientific rationale for conducting such analyses. Analyses were conducted in R (version 4.2.2) and SAS (version 9.4); all p-values are two-sided.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Data Availability

The dataset supporting the findings of this study has been deposited in Zenodo and is publicly available online at <https://zenodo.org/records/10035257> and <https://stjude.cloud>. Source data for Figs. 1 and 2 have been provided as Source Data files. The DNA methylation data used in this study are accessible at the NCBI GEO website under the accession number GSE197674 for survivors and GSE197676 for controls. All other data supporting the findings of this study are available from the co-corresponding authors on reasonable request.

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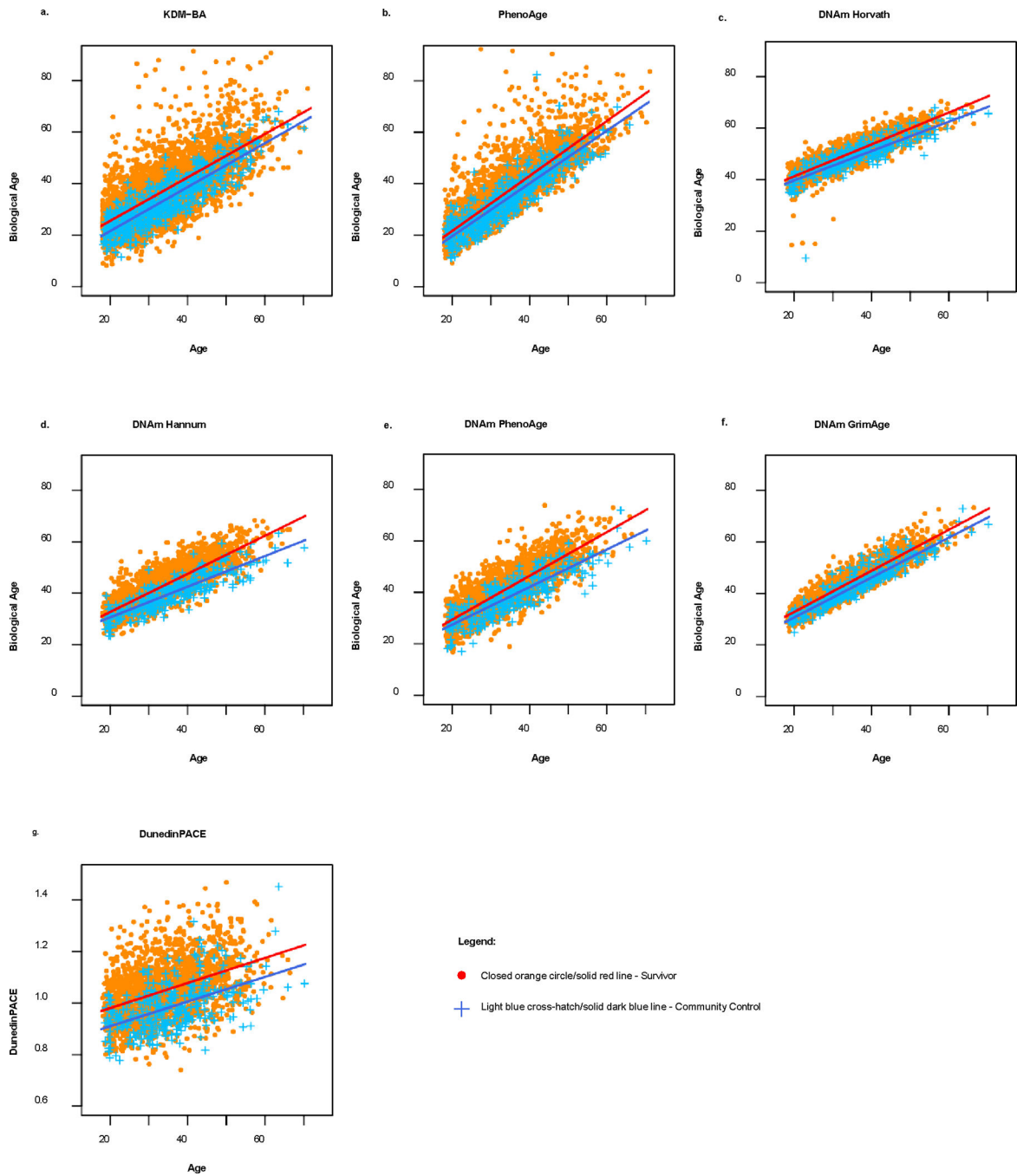


Fig. 1: Scatterplots and linear regression lines of biological age by chronological age (i.e., age at DNA sampling) for childhood cancer survivors (N = 4117) and community controls (N = 606) in the St. Jude Lifetime Cohort Study.

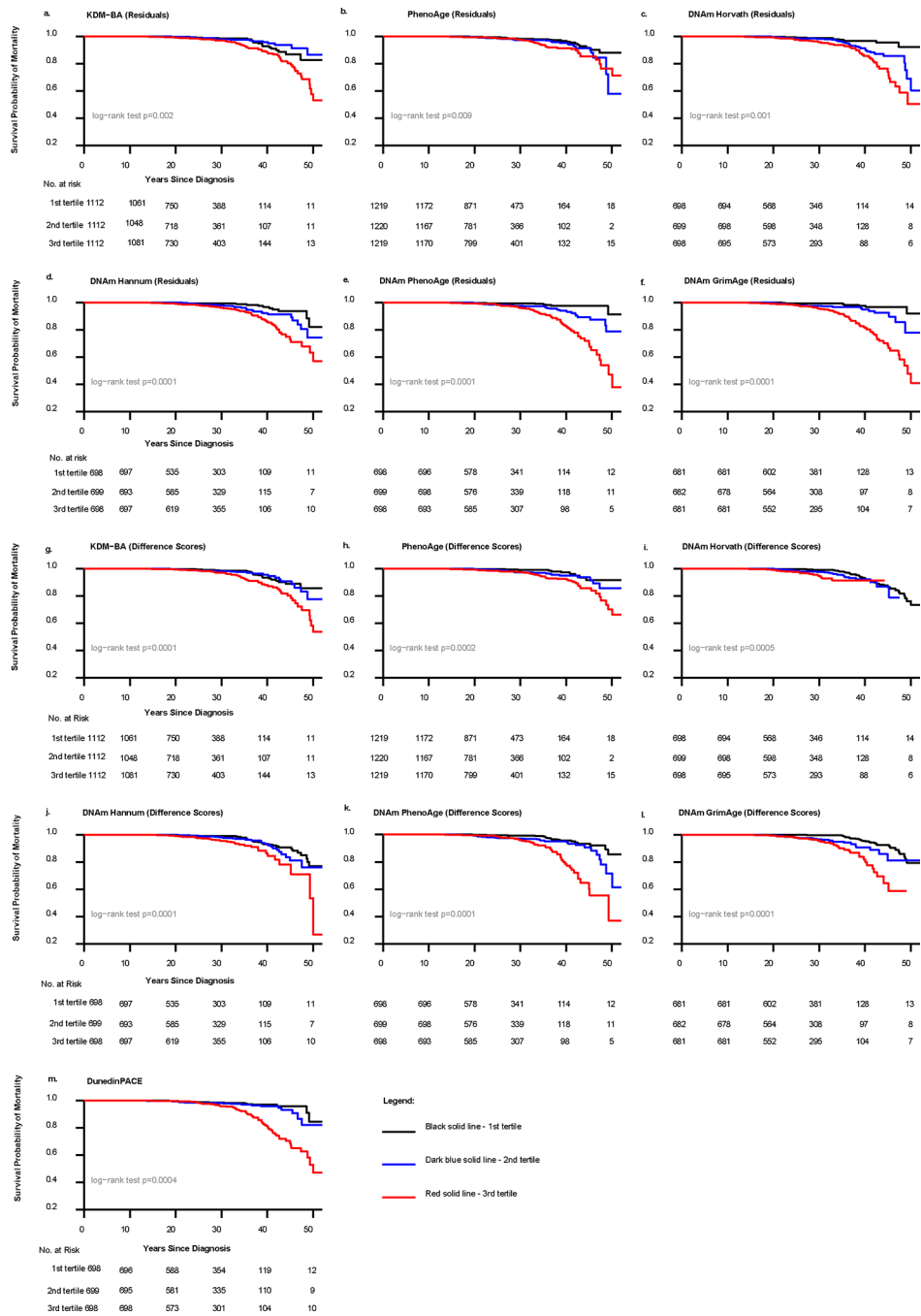


Fig. 2: Kaplan-Meier survival curves of biological age acceleration and all-cause mortality among St. Jude Lifetime Cohort Study childhood cancer survivors (N = 4117). Note: all models are unadjusted and the sample sizes analyzed (n = (2,044, 3,658)) differ by biological age measure.

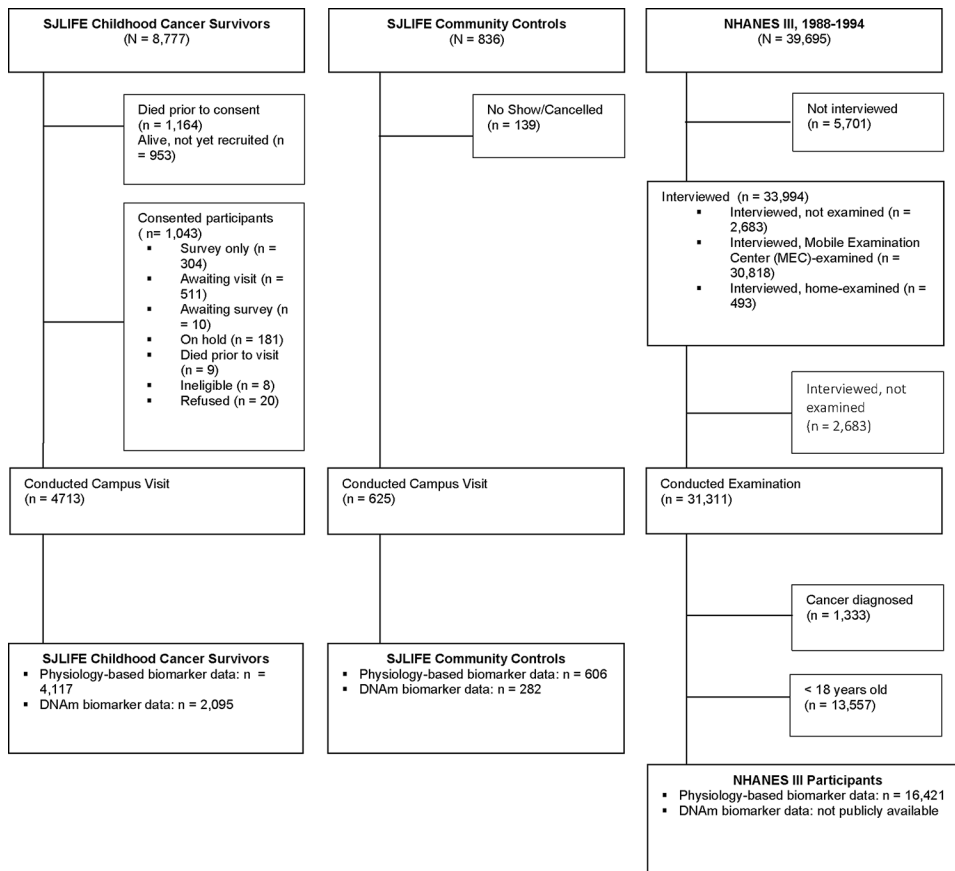


Fig. 3: Consort diagram of the St. Jude Lifetime (SJLIFE) Cohort Study childhood cancer survivors (N = 8,777) and community controls (N = 836), and the National Health and Nutrition Examination Survey (NHANES) III (N = 39,695) study samples.

Table 1.

Clinical and Demographic Characteristics of Participants and Controls

	SJLIFE Survivors		SJLIFE Community controls		NHANES controls		p-value
	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	
Age at evaluation, years	4,117	35.08 (10.17)	606	33.64 (10.18)	16,421	42.07 (13.11)	<0.001
Biological Age							
Klemera-Doubal Method	4,117	37.87 (13.10)	606	33.17 (9.99)	16,421	41.61 (12.63)	<0.001
Phenotypic Age	4,117	36.95 (12.76)	606	33.87 (11.41)	16,421	41.96 (13.93)	<0.001
Horvath's Clock	2,095	49.25 (6.74)	282	49.08 (6.82)	-	-	0.69
Hannum's Clock	2,095	42.56 (8.44)	282	40.05 (7.09)	-	-	<0.001
DNAmPhenoAge Clock	2,095	40.76 (9.82)	282	39.05 (8.71)	-	-	0.01
GrimAge Clock	2,095	42.81 (7.98)	282	43.30 (8.60)	-	-	0.34
DunedinPACE	2,095	1.05 (0.11)	282	0.99 (0.10)	-	-	<0.001
	N = 4,117	%	N = 606	%	N = 16,421	%	
Sex							<0.001
Male	2,169	52.68	266	43.89	7,541	48.29	
Female	1,948	47.32	340	56.11	8,524	51.71	
Race/ethnicity							
Non-Hispanic White	3,311	80.42	517	85.31	5,960	74.34	-
Non-Hispanic Black	639	15.52	40	6.60	4,789	11.78	
Hispanic	111	2.70	22	3.63	5,316	13.88	
Asian	8	0.19	14	2.31	-	-	
Other	45	1.09	7	1.16	262	3.91	
Unknown	3	0.07	6	0.99	-	-	
Educational attainment							<0.001
< High School	443	11.09	17	2.91	6,571	25.04	
High School Graduate	2,329	58.28	262	44.86	7,542	55.15	
College Graduate	1,224	30.63	305	52.23	1,841	19.81	
Insurance status							0.03
Yes	3,353	83.97	515	87.88	12,435	85.02	
No	640	16.03	71	12.12	3,251	14.98	
Annual household income							<0.001
< \$40,000	1,449	41.31	132	24.22	11,235	64.36	
\$40,000	2,059	58.69	413	75.78	3,281	35.64	
BMI							<0.001
Underweight	139	3.38	15	2.48	376	2.63	
Normal	1,262	30.66	228	37.62	6121	43.44	
Overweight	1,171	28.45	169	27.89	5441	32.02	
Obese	1,544	37.51	194	32.01	4078	21.91	
Depression score, mean (SD)	49.84	10.34	47.31	8.73	-	-	<0.001

	SJLIFE Survivors		SJLIFE Community controls		NHANES controls		p-value
	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	
Smoking status							<0.001
Never	2,821	70.19	419	70.66	8,184	47.32	
Former	484	12.04	101	17.03	3,625	23.66	
Current	714	17.77	73	12.31	4,255	29.02	
Physical Activity (≥ 150 weekly minutes of MVPA)							<0.001
Yes	2,109	52.71	363	61.84	8,641	62.61	
No	1,892	47.29	224	38.16	7,418	37.39	
Alcohol Use							<0.001
Risky drinking	1,882	47.07	347	58.81	4,142	27.24	
No risky drinking	2,116	52.93	243	41.19	11,370	72.76	

SD=standard deviation, MVPA=moderate or vigorous physical activity

Table 2.

Unstandardized Differences in Biological Age Acceleration among Adult Survivors of Childhood Cancer in the St. Jude Lifetime Cohort, Community Controls, and NHANES III Participants

Physiology-Based Measures	BAA Estimated with Residuals				BAA Estimated with Difference Scores			
	SJLIFE Participants (N=4,117)		Community Controls (N=606)		SJLIFE Participants (N=4,117)		Community Controls (N=606)	
	Mean (SD)	Mean (SD)	Mean Difference	95% CI	Mean (SD)	Mean (SD)	Mean Difference	95% CI
Klemera-Doubal Method	0.98 (0.17)	0.38 (0.40)	0.60	(-0.25, 1.45)	6.04 (0.17)	-0.45 (0.39)	6.49	(5.67, 7.31)
Phenotypic Age	0.53 (0.13)	-0.07 (0.35)	0.59	(-0.14, 1.34)	5.21 (0.13)	-0.01 (0.35)	5.22	(4.49, 5.94)
DNA Methylation-Based Measures	N = 2,095	N = 282			N = 2,095	N = 282		
Horvath's Clock	2.29 (0.08)	0.41 (0.21)	1.88	(1.45, 2.31)	15.92 (0.07)	12.35 (0.19)	3.57	(3.17, 3.97)
Hannum's Clock	1.42 (0.10)	0.33 (0.27)	1.09	(0.52, 1.66)	9.22 (0.10)	3.57 (0.27)	5.65	(5.08, 6.21)
DNAmPhenoAge Clock	0.99 (0.12)	0.20 (0.32)	0.79	(0.12, 1.46)	7.41 (0.12)	2.79 (0.32)	4.62	(3.95, 5.29)
GrimAge Clock	1.29 (0.07)	0.17 (0.19)	1.12	(0.72, 1.51)	9.18 (0.07)	6.96 (0.18)	2.22	(1.83, 2.60)
DunedinPACE*	1.04 (0.002)	0.99 (0.01)	0.05	(0.04, 0.06)				
Physiology-Based Measures	SJLIFE Participants (N=4,117)		NHANES III Controls (N=16,421)		SJLIFE Participants (N=4,117)		NHANES III Controls (N=16,421)	
	Mean (SD)	Mean (SD)	Mean Difference (β)	95% CI	Mean (SD)	Mean (SD)	Mean Difference (β)	95% CI
	0.98 (0.12)	0.23 (0.06)	0.75	(0.49, 1.02)	6.04 (0.12)	1.10 (0.06)	4.94	(4.68, 5.20)
Phenotypic Age	0.53 (0.12)	-0.33 (0.06)	0.86	(0.59, 1.13)	5.21 (0.12)	0.40 (0.06)	4.81	(4.54, 5.08)

BAA = Biological Age Acceleration; Mean = Biological age acceleration mean; SE = Standard Error; β = Beta Coefficient (Mean Difference); 95% CI = 95% Confidence Interval

* For DunedinPACE, the non-residualized values (original scale) were used.

Biological age acceleration estimated with residuals was calculated by regressing biological age on chronological age. Biological age acceleration estimated with difference scores was calculated by subtracting chronological ages from the estimated biological ages. For DunedinPACE, the non-residualized values (original scale) were used. Linear regression models were used to estimate the mean difference between survivors and controls; models are unadjusted. The unstandardized mean difference for all biological age measures (except DunedinPACE) represents the total lifespan difference in aging in survivors compared to controls. The interpretation for DunedinPACE is different because it is a rate; the unstandardized mean difference can be interpreted as the difference in how much aging occurs per year in survivors compared to controls.

Table 3.

Adjusted Associations of Biological Age Acceleration by Tertile with Frailty and All-Cause Mortality among Survivors

Biological Age Acceleration Calculated with Residuals									
	Tertile	n	Frailty (Ref = robust)				All-Cause Mortality		
			Pre-Frail (n = 2,307)		Frail (n = 287)		(n = 167 deaths)		
			HR	95% CI	HR	95% CI	n	HR	95% CI
Klemera-Doubal Method	1	3,298	Ref		Ref		3,336	Ref	
	2		1.04	0.83, 1.31	1.36	1.06, 1.74		1.14	0.59, 2.20
	3		1.42	0.90, 2.24	2.22	1.38, 3.55		3.38	1.94, 5.89
Phenotypic Age	1	3,615	Ref		Ref		3,658	Ref	
	2		1.09	0.88, 1.35	1.39	1.10, 1.75		1.59	0.89, 2.84
	3		0.71	0.46, 1.10	1.6	1.06, 2.43		1.77	1.02, 3.08
Horvath's Clock	1	2,081	Ref		Ref		2,095	Ref	
	2		1.58	1.19, 2.08	1.27	0.96, 1.67		2.8	1.39, 5.65
	3		2.32	1.30, 4.14	2.01	1.13, 3.58		4.89	2.50, 9.58
Hannum's Clock	1	2,081	Ref		Ref		2,095	Ref	
	2		1.02	0.78, 1.33	1.2	0.92, 1.58		1.7	0.83, 3.50
	3		0.9	0.52, 1.55	1.25	0.73, 2.14		3.58	1.89, 6.80
DNAmPhenoAge Clock	1	2,081	Ref		Ref		2,095	Ref	
	2		1.19	0.91, 1.55	1.44	1.09, 1.90		2.57	1.18, 5.63
	3		1.66	0.94, 2.96	2.35	1.35, 4.10		5.09	2.47, 10.50
GrimAge Clock	1	2,035	Ref		Ref		2,044	Ref	
	2		1.19	0.90, 1.56	1.75	1.28, 2.39		3.01	1.28, 7.07
	3		2.54	1.40, 4.59	3.13	1.63, 6.03		7.59	3.36, 17.16
Biological Age Acceleration Calculated with Difference Scores									
Klemera-Doubal Method	1	3298	Ref		Ref		3336	Ref	
	2		1.08	0.86, 1.35	1.37	1.07, 1.75		2	1.09, 3.67
	3		1.38	0.88, 2.17	1.97	1.24, 3.13		4.57	2.62, 7.99
Phenotypic Age	1	3,615	Ref		Ref		3,658	Ref	
	2		0.85	0.69, 1.06	1.23	0.97, 1.55		1.79	0.88, 3.64
	3		0.52	0.33, 0.83	1.72	1.13, 2.61		2.8	1.44, 5.45
Horvath's Clock	1	2,081	Ref		Ref		2,095	Ref	
	2		1.15	0.87, 1.52	1.11	0.83, 1.50		4.86	2.55, 9.28
	3		1.46	0.84, 2.56	1.43	0.80, 2.58		14.54	6.47, 32.68
Hannum's Clock	1	2,081	Ref		Ref		2,095	Ref	
	2		0.99	0.75, 1.30	1.15	0.87, 1.53		1.59	0.90, 2.81
	3		0.91	0.53, 1.56	1.08	0.62, 1.87		4.78	2.71, 8.44
DNAmPhenoAge Clock	1	2,081	Ref		Ref		2,095	Ref	
	2		1.04	0.80, 1.36	1.39	1.05, 1.84		2.41	1.29, 4.50

Biological Age Acceleration Calculated with Residuals									
	Tertile	n	Frailty (Ref = robust)				All-Cause Mortality		
			Pre-Frail (n = 2,307)		Frail (n = 287)		(n = 167 deaths)		
			HR	95% CI	HR	95% CI	n	HR	95% CI
GrimAge Clock	3		1.35	0.76, 2.39	2.33	1.34, 4.07		4.8	2.65, 8.71
	1	2,035	Ref			Ref	2,044	Ref	
	2		1.13	0.85, 1.49	1.34	0.98, 1.83		3.07	1.53, 6.15
	3		1.38	0.77, 2.46	2.38	1.28, 4.44		9.42	4.74, 18.75
DunedinPACE	1	2,081	Ref			Ref	2,094	Ref	
	2		1.05	0.82, 1.35	1.5	1.06, 2.13		0.84	0.43, 1.68
	3		1.28	0.74, 2.19	2.61	1.39, 4.89		2.36	1.25, 4.46

OR = Odds ratio, HR = Hazard ratio, 95% CI = 95% Confidence Interval

Note: Frailty and mortality are the outcome variables, and biological age acceleration by tertile is the independent predictor of interest. Frailty models were estimated with multinomial logistic regression using the "robust" category as the reference group. All-cause mortality models were estimated using Cox regression models. Models are adjusted for age at diagnosis, race/ethnicity, sex, treatment type (chemotherapy, radiotherapy, surgery), BMI, depression, smoking, alcohol use, and physical activity.