Examples of Funded Grants in Behavioral Research

Overview
The National Cancer Institute (NCI) frequently receives requests for examples of funded grant applications. Several investigators and their organizations agreed to let the Behavioral Research Program (BRP) post excerpts of their grant applications online.

About
We are grateful to the investigators and their institutions for allowing us to provide this important resource to the research community. To maintain confidentiality, we have redacted some information from these documents (e.g., budgets, social security numbers, home addresses, introduction to revised application), where applicable. In addition, we only include a copy of SF 424 R&R Face Page, Project Summary/Abstract (Description), Project Narrative, Specific Aims, and Research Strategy; we do not include other SF 424 (R&R) forms or requisite information found in the full grant application (e.g., performance sites, key personnel, biographical sketches).

Accessibility
Individuals using assistive technology (e.g., screen reader, Braille reader) who experience difficulty accessing any information should send an email to the Behavioral Research Program (ncidccpsbrpadvances@mail.nih.gov).

424 R&R and PHS-398 Specific
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SF 424 R&R Face Page

PI: Irwin, Michael R

Grant Number: 1 R01 CA207130-01

Title: Sleep Disturbance, Inflammation, and Cellular Aging in Breast Cancer Survivors

FOA: PA13-302

FOA Title: RESEARCH PROJECT GRANT (PARENT R01)

Organization: UNIVERSITY OF CALIFORNIA LOS ANGELES

Department: NPI Semel Institute

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Organization: NPI Semel Institute, Kaiser Permanente Southern California

Role Category: PD/PI

Project Summary/Abstract

Advances in cancer treatment have resulted in a growing number of cancer survivors in the United States. Despite the success of cancer treatments, survivors face long-term changes in health, with twice the likelihood of disability as those without a cancer history, greater risk for second primary cancers, more age-related comorbid disorders, and 28% reduction in life expectancy. This study hypothesizes that the increased risk of morbidity and mortality in cancer survivors is due to accelerated biological aging. Furthermore given that the risk for the late effects of cancer diagnosis and treatment show considerable variability, individual differences may either confer protection or promote vulnerability. Given our preliminary data that sleep disturbance leads to greater increases in inflammation and telomere erosion over a one year period, we further hypothesize that sleep disturbance and depression history serve as susceptibility factors to accelerate biological aging. To examine these questions, this study leverages an existing project (CA160245) of a Kaiser Permanente Southern California (KPSC) SEER-affiliated tumor registry-based sample of 300 (>55 years) breast cancer survivors, includes biological aging outcomes of cellular and transcriptional markers of inflammation and telomere erosion, and recruits a KPSC comparison cohort of 300 older women without a cancer history. Both KPSC groups will be examined at baseline and prospectively followed at 8, 16, 24, and 32 months to address three specific aims: 1) to examine differences at baseline and in prospective rate of change of cellular and transcriptional markers of inflammation and telomere length as a function of breast cancer survivorship; 2) to examine differences at baseline and in prospective rate of change of cellular and transcriptional markers of inflammation and telomere length as a function of sleep disturbance and breast cancer survivorship; 3) to examine differences at baseline and in prospective rate of change of cellular and transcriptional markers of inflammation and telomere length as a function of depression history and breast cancer survivorship
This study will determine whether biological aging is driven by breast cancer status, by independent effects of sleep disturbance or depression history, or by the interaction of these behavioral factors with breast cancer status. Such information is necessary to define the risk population (i.e., breast cancer survivors, depression history) and/or risk factors (i.e., sleep disturbance) in the design, implementation, and delivery of treatments, which selectively target biological aging with greatest efficacy with the potential to reduce risk of age-related morbidities.

Relevance to Public Health

In line with the Precision Medicine Initiative, this study identifies the individual factors that contribute to biological aging in breast cancer survivors vs. comparison women, and determines whether sleep disturbance and depression history are susceptibility factors that independently increase biological aging and whether these behavioral vulnerabilities interact with breast cancer status to accelerate biological aging. These findings have implications for predicting cancer and non-cancer morbid outcomes, and for the development of interventions (i.e., insomnia treatment) that can target vulnerable groups (i.e., depression history) with biological precision (i.e., elevated levels of inflammation) to mitigate age-related morbidity associated with these biomarker risk profiles in breast cancer survivors as compared to older adult women.

Specific Aims

Advances in cancer treatment have resulted in a growing number of cancer survivors in the United States; for breast cancer survivors, 5-year survival rates are over 90% with nearly 3 million breast cancer survivors.[1-3] Despite the success of cancer treatments, survivors face long-term changes in health, with twice the likelihood of disability as those without a cancer history,[1,2] greater risk for second primary cancers, more age-related comorbid disorders (e.g., cardiovascular disease, diabetes), and 28% reduction in life expectancy.[3-5] Biological aging, as measured by increased inflammation and telomere length attrition (i.e., telomere erosion), predicts morbidity and mortality independent of chronological age,[6-8] with similar evidence in cancer survivors.[9,10] Hence, we hypothesize that the increased risk of morbidity and mortality in cancer survivors is due to accelerated biological aging. Indeed, in cross-sectional analyses of breast cancer survivors vs. women without cancer history (n=304; 55-80 y), we found accelerated age-related increases in inflammation, along with shortening of peripheral blood mononuclear cell (PBMC) telomere length. This study uses a longitudinal prospective design, to test whether older adult breast cancer survivors show accelerated biological aging as compared to matched women without a cancer history.

Risk for the late effects of cancer diagnosis and treatment show considerable variability, suggesting that individual differences provide either protection or promote vulnerability. Both sleep disturbance and depression history are independent predictors of all-cause mortality,[11-13] and this study further hypothesizes that sleep disturbance and depression history serve as susceptibility factors to accelerate biological aging. Indeed, our preliminary data show that sleep disturbance activates inflammatory signaling;[14-17] insomnia is associated with greater age-related shortening of telomere length; sleep disturbance and depression history together predict greater increases in inflammation and telomere erosion; and unremitting insomnia leads to greater increases in inflammation and telomere erosion over a one year period. Given that breast cancer survivors show an increased prevalence of sleep disturbance and depression history,[2,18-22] this study will recruit a group of matched women without a cancer history who have comparable prevalence of sleep disturbance and depression history, balance the groups for these risk factors, and test whether sleep disturbance and depression history have independent effects or interact with breast cancer status to accelerate biological aging.

Specifically, we will leverage an existing project (R01 CA160245) that is prospectively examining sleep disturbance and depression risk in a Kaiser Permanente Southern California (KPSC) SEER-affiliated tumor registry-based sample of 300 breast cancer survivors (>55 y) with 32 months follow-up. We will recruit a matched comparison cohort of 300 older women without a cancer history who are also KPSC members and who have comparable prevalence of sleep disturbance and depression history to address the following aims:

1. **To examine differences at baseline and in prospective rate of change of cellular and transcriptional markers of inflammation and telomere length as a function of breast cancer survivorship.** Breast cancer survivors will show increases in inflammation and telomere attrition at baseline and greater rates of increase of inflammation and telomere erosion as compared to matched older adult women without cancer.
2. **To examine differences at baseline and in prospective rate of change of cellular and transcriptional markers of inflammation and telomere length as a function of sleep disturbance and breast cancer survivorship.** Sleep disturbance will be independently associated with increases in inflammation and telomere attrition at baseline and greater rates of increase in markers of inflammation and telomere erosion. Sleep disturbance will interact with breast cancer survivor status to accelerate biological aging.
3. **To examine differences at baseline and in prospective rate of change of cellular and transcriptional markers of inflammation and telomere length as a function of depression history and breast cancer survivorship.** Depression history will be independently associated with increases in inflammation and telomere attrition at baseline and greater rates of increase in markers of inflammation and telomere erosion. Depression history will interact with breast cancer survivor status to accelerate biological aging.

Exploratory aim: To examine differences in inflammation and telomere attrition as a function of baseline characteristics, and interactions between sleep disturbance, depression history, and breast cancer status.

Impact Statement: This project will be the first to examine prospectively whether rates of change of markers of biological aging differ between breast cancer survivors and matched women without a cancer history, and to what extent sleep disturbances and depression history serve as independent risk factors or as susceptibility factors for accelerated aging in breast cancer survivors. *Hence, this study will determine whether biological aging is driven by breast cancer status, by independent effects of sleep disturbance or depression history, or by the interaction of these behavioral factors with breast cancer status*. Such information is necessary to define the risk population (i.e., breast cancer survivors, depression history) and/or risk factors (i.e., sleep disturbance) in the design, implementation, and delivery of treatments, which selectively target biological aging and risk of age-related morbidities with greatest efficacy (i.e., number needed to treat, NNT).

Research Strategy

A. Significance

A.1. Cancer Survivorship and Accelerated Aging: Public Health Significance

In the United States, over 13.7 million people are cancer survivors,[23] and nearly 65% of cancer survivors will be 65 y or older by 2020.[23,24] Cancer survivors are at increased risk of morbidity and mortality,[3-5] have twice the likelihood of poor health and disability as individuals who do not have a cancer history,[1] show greater risk for second primary cancers as well as a number of age-related comorbid disorders with related poorer functioning,[3,4] and have a lower life expectancy of 10.4 years due to cancer relapse and of 6.2 years due to other causes[25] leading to an overall reduction in life expectancy of 28%.[3,4] Together, these data raise the question that cancer diagnosis and treatment, even focal radiotherapy,[26] may accelerate the aging process that increases risk for secondary health issues, referred to as the "late effects" of cancer treatment.

This study will address this significant question by enrolling both breast cancer survivors and comparison women without cancer who have similar healthcare access via KPSC. A comparison group is necessary to understand if breast cancer survivors evidence accelerated biological aging relative to women without cancer.

A.2. Cancer Survivorship, Inflammation, and Telomere Biology

Inflammation: Inflammation increases with age, is prognostic for numerous age-related disorders and mortality, including several human cancers,[27] and is potentially linked to 20% of all cancer deaths worldwide.[28] Inflammation is also associated with cancer recurrence,[9] and secondary health issues (i.e., cardiovascular disease,[29] leading to the hypothesis suspect that increased inflammation contributes to late effects of cancer.

Telomere biology: Telomere length, a repeat sequence of DNA that caps the end of chromosomes, is a biomarker of cellular aging,[30] which correlates with biological aging. Telomere biology is prognostic for cancer specific survival[31,32] and all-cause mortality,[13] in which shorter telomere length prospectively predicts reduced 20 year survival after any cancer (n=47,102).[31] Furthermore, in breast cancer survivors, telomere erosion over 2 years predicts increased mortality risk[10,33] including cardiovascular mortality.[34]

There is an absence of prospective data to determine whether breast cancer survivors show accelerated increases in inflammation and telomere attrition as compared to comparison women. Results of this study are significant in development of clinical care guidelines to monitor these markers of biological aging in breast cancer survivors, and in development of selective prevention strategies that target breast cancer survivors to reverse or prevent increases in these proximal indicators of morbidity such as breast cancer recurrence.[35]

A.3. Sleep Disturbance, Inflammation, and Telomere Biology in Breast Cancer Survivors

Cancer survivors have a 2- to 4 fold increase in the prevalence of insomnia symptoms compared to healthy adults,[18] with 51% reporting difficulties sleeping during the cancer survivorship period,[18] in which high rates of sleep complaints persist even several years after the end of adjuvant endocrine therapy for breast cancer, especially in older women.[18] Given that sleep disturbance is an independent predictor of all-cause mortality,[36-40] sleep behaviors may play an independent role in biological aging and also influence risk for "late effects."

Inflammatory Biology: Sleep disturbance leads to daytime increases in inflammation,[41-43] which are associated with fatigue in breast cancer survivors.[44] Even modest sleep loss activates cellular inflammation[14] and inflammatory signaling (e.g., NF-κB)[15] with greater increases in women, and with a depression history.

Telomere Biology: Poor sleep quality, as well as short sleep duration,[45,46] are linked to shorter leukocyte telomere length in women,[47] and older adults.[48] Finally, persistent sleep disturbance (i.e., night shifts over years) is associated with shorter telomere length in a dose-response manner.[46] Additionally, adequate sleep has been found to preserve telomere length or mitigate telomere shortening due to age or chronic disease.[49]

Virtually no prospective data link sleep disturbance to increases inflammation or telomere attrition. This study is significant by testing whether sleep disturbance has independent effects and/or interacts with breast cancer status to amplify rates of biological aging. (Increased prevalence of sleep disturbance in breast cancer survivors will be experimentally balanced by identifying a group of matched women

without a cancer history who have comparable prevalence of sleep disturbance.) If insomnia uniquely predicts biological aging independent of breast cancer status, or primarily interacts with breast cancer status, understanding the relative effects sizes linking sleep disturbance to markers of biological aging in breast cancer survivors vs. comparison women is critical in the development of a precision medicine prevention trial that "universally" treats insomnia to mitigate inflammation or telomere erosion (as we have preliminarily demonstrated[50]), or that selectively targets breast cancer survivors with insomnia to enhance trial efficacy.

A.4. Depression, Inflammation, and Telomere Biology in Breast Cancer Survivors

Major depression occurrence in cancer survivors is 22-29%, 3-4 times higher than the incidence in adults.[2,17] As the population ages and the number of cancer survivors grows dramatically, depression is projected to increase by 2030 to be the greatest contributor to illness burden.[51] Furthermore, depression is a recurrent disorder in which over 75% have two or more episodes. Indeed, those with a prior depression history are more vulnerable to the effects of cancer diagnosis and treatment, show greater declines in physical functioning from pre- to post-chemotherapy than women without history, are greater risk for sustained distress following treatment,[52] and have 39% higher all-cause mortality than non-depressed patients,[53] whether depression occurred before or after cancer.[12]

Inflammatory Biology: Experimental- and prospective data show that levels of inflammation lead to increases in depressive symptoms and depression,[54] especially in high risk populations such as breast cancer survivors.[2,55] Acute treatment with chemotherapy activates inflammatory signaling, which is associated with depression in the immediate post-treatment period (i.e., 6 months) in breast cancer patients;[56] Inflammation may also be a consequence of depression, as cumulative depression episodes predict subsequent CRP levels.[57] Furthermore, prior depression plays a sensitizing role in the promotion of stress-related inflammatory responses,[58] which could promote cellular aging.[32]

Telomere Biology: In breast cancer survivors, high psychological stress is associated with shorter leukocyte telomere length,[10] similar to findings in female caregivers[59] and in otherwise healthy women.[60] Breast cancer survivors who have a depression history are more likely to experience recurrent depression,[22] and may be more vulnerable to telomere erosion. Depression[61,62] and especially number of depressive episodes are associated a greater load of short telomeres[63,64] independent of antidepressant use.[65] Moreover, two longitudinal studies,[64,66] but not another,[67] have found that depressive symptoms predict shorter telomere length. No study has characterized the prospective association between depressive episodes, depression history, and telomere erosion rates in breast cancer survivors versus comparison women.

By characterizing the contribution of sleep disturbance and depression history on inflammation and telomere erosion, development of a prevention trial will be refined; targeting two or more risk factors can optimize health gains in those most vulnerable, with a substantial drop in the NNT.

A.5. Summary of Significance

Inflammation and telomere erosion are highly correlated with clinically relevant outcomes, including breast cancer recurrence. Consistent with the goals of precision medicine, this study will define the prospective impact of individual risk factors (i.e., breast cancer- vs. non-cancer status, sleep disturbance, depression history) and/or their interaction on rate of change of biological aging with implications for "precision targeting" to prevent of age-related disease and cancer. Hence, defining the relative impact of these risk factors on rate of change of markers of biological aging will guide selective identification of the risk population (i.e., breast cancer survivors, those with depression history) as well as the target for intervention (i.e., sleep disturbance). First, understanding the magnitude of prospective changes in markers of biological aging informs the development of strategies to monitor biological aging, design of a prevention trial (i.e., power estimates based on prospective change), and trial efficacy (i.e., selectively targeting a population or modifiable risk factor) to prevent increases in markers of biological aging (i.e, lowering NNT). For example, if breast cancer status uniquely contributes to biological aging, and inflammation moderates this trajectory, then a rationale would be supported for breast cancer survivors to be prioritized over women without cancer, possibly for treatment with anti-inflammatory medications or other interventions (i.e., diet, exercise) that reduce inflammation. Secondly, if sleep disturbance is found to have an independent impact on biological aging in the non-cancer group and this relative risk is greater than breast cancer status as our preliminary data suggest, then a rationale would be supported for prioritizing all women with insomnia for treatment (i.e., CBT-I); such treatment would be

implemented “universally” in both breast cancer- as well as non-cancer groups. Although we have found that insomnia treatment reduces inflammation, this study will provide evidence about the importance of insomnia as a priority target to reduce biological aging, relative to other risk factors. Thirdly, if breast cancer status, depression history, and sleep disturbance interact to predict accelerated biological aging, then a rationale would be supported to target a selective risk group with “two hits” (i.e., breast cancer status, depression history) for treatment of the “third hit” insomnia, leading to substantial reduction in NNT (i.e., 30 to 4) to reverse biological aging. Fourthly, if inflammation is found to moderate biological aging in this high risk group with “three hits”, a rationale would be further supported for testing whether medications or other interventions that attenuate inflammation might bolster the efficacy of behavioral interventions that solely target sleep disturbance or depression. Finally, we are singly positioned to translate our findings given KPSC’s integrated health care delivery system, which can rapidly implement interventions via population care management to promote the optimal use of prevention approaches for cancer survivors or non-cancer older adults, both by primary and specialty care clinicians. Additionally, via electronic medical records, KPSC is able to monitor safety of interventions and track long-term clinical outcomes that relate to a reduced rate of biological aging.

B. Innovation

This research is innovative by including patient reported outcomes to test a biobehavioral framework, which has the potential to translate into targeted behavioral interventions that mitigate accelerated age-related increases in inflammation and telomere erosion in breast cancer survivors vs. a comparison cohort. No prior research has tested whether breast cancer survivors show accelerated aging, nor prospectively evaluated the rate of change of inflammation and telomere attrition in cancer survivors as compared to older adults who are aging without a cancer diagnosis. In addition, the proposed research has important implications for examining behavioral pathways that contribute to aging, and whether sleep and depression history have independent effects of interact with cancer diagnosis to accelerate increases in biomarkers of aging.

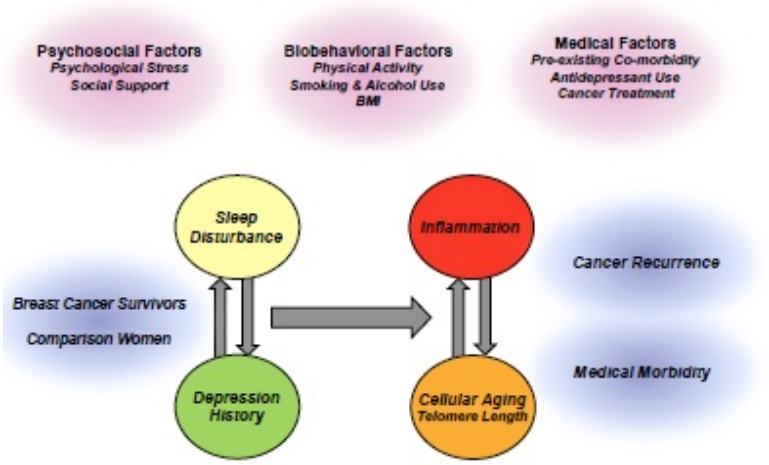


Figure 1: Model depicting the impact of sleep disturbance and depression history on inflammation and cellular aging in breast cancer survivors and comparison women, taking into account the relationships with inflammation and psychosocial-, biobehavioral-, and medical factors. This model has implications for cancer recurrence and medical morbidity.

Understanding the contributions of sleep disturbance and depression history to the differential biologic risk profiles of inflammation and telomere erosion in breast cancer survivors vs. a comparison cohort is the first step in the conceptual rationale, justification, and power estimates to build an efficient prevention trial with optimal efficacy to prioritize selectively targeting breast cancer survivors, those with insomnia or depression history, or those breast cancer survivors with sleep disturbance and/or depression history.

B.1. Provides novel insight into the selective risk indicators of inflammation and telomere erosion in breast cancer survivors, and differential prospective changes in these markers of aging Enhanced proinflammatory signaling is one proposed pathway through which senescent cells are involved in the aging process and cancer risk.[6] Alternatively, the rise in inflammation with increasing chronological age may play a role in cellular aging of the immune system.[68] Given that aging immune cells have a heightened proinflammatory response, a feed forward increase in inflammation and cellular aging may occur. *However, no prospective study has evaluated the reciprocal temporal profiles of inflammation and telomere erosion in older adults, and whether these prospective trajectories are altered in breast cancer survivors as compared to older adult women.* Hence, this study is highly innovative by examining differential prospective trajectories of change in inflammation and telomere erosion in breast cancer survivors vs. comparison women.

B.2. Promotes a paradigm shift into the role of behavioral factors that contribute to age-related accelerated increases in inflammation and telomere erosion, and the role of sleep disturbance and depression on accelerated biological aging in breast cancer survivors. Given that cancer specific factors (i.e., tumor type, treatment) do not alter the associations between inflammation and telomere erosion and prediction of breast cancer recurrence and mortality,[9,33] efforts are needed to understand why this risk exists

and what can be done to ameliorate. If the roles of sleep disturbance and/or depression history on differential age-related increases in inflammation and telomere erosion in breast cancer survivors are confirmed, these novel prospective data would provide a compelling argument for the development of highly specific interventions that target this modifiable risk factor (i.e., sleep disturbance) and its occurrence in a vulnerable population (i.e., depression history) to mitigate these biological risk profiles. *Further, without comparative prospective data in women without cancer, it is not possible to know whether breast cancer survivors show accelerated aging, and to what extent sleep disturbance and depression history interact with cancer specific factors to contribute to this risk.* Understanding the relative risk profiles promotes the development and design of selective pragmatic prevention trials with accurate power estimates to reduce rates of biological aging.

B.3. Fosters translational research to identify links between sleep, depression, inflammation and aging biology, and the unique differences found in cancer survivors. The proposal extends recent advances in inflammatory and aging biology to better understand the behavioral factors that influence accelerated aging, which may be unique to cancer survivors due to either increased prevalence or interaction of these behavioral mechanisms with cancer diagnosis and treatment. Such findings would further support the rationale for testing whether medications or other interventions (e.g., exercise) might reverse inflammation and cellular aging, especially in those with elevated inflammation (i.e., biological precision), and thereby enhance the efficacy of behavioral treatments that solely target sleep disturbance or depression. Further, these prospective data are hypothesized to yield differential effect sizes, which will guide precision medicine efforts to determine NNT in breast cancer survivors, as compared to those without cancer, necessary for selective prevention efforts.

C. Approach

C.1. Preliminary Studies

This proposed prospective cohort study is a companion to an ongoing project (CA160245) that is examining the prospective association between sleep disturbance, inflammation and depression risk in a KPSC, population based cohort of 300 breast cancer survivors (>55 years) identified from the KPSC-SEER affiliated tumor registry. Although CA160245 originally proposed enrollment of 300 matched comparison women (i.e., as shown in NIH Reporter abstract), this comparison group was removed at the time of funding. Hence, the present study will build upon the infrastructure of an ongoing collaborative project between UCLA and KPSC that has enrolled only breast cancer survivors, and will newly recruit and follow 300 matched comparison women from the same KPSC health plan; the comparison women do not have cancer but will have comparable prevalence of sleep disturbance and depression history. This companion study, as proposed, will provide an opportunity to ask unique questions about prospective differences in the rate of change of inflammation and telomere erosion in breast cancer survivors vs. the comparison cohort, and the differential contribution of sleep disturbance and depression history to this risk profile in these two groups. None of these aims were proposed in CA160245. This companion study is also significant for the goals of CA160245, as it will restore 300 matched comparison women to the original study design and provide an opportunity to evaluate the differential risk profile of sleep disturbance and depression in breast cancer survivors vs. older adult women without cancer. CA160245 has enrolled 315 breast cancer survivors with a >95% retention in the ongoing follow-up.

C.1.1 Is breast cancer survivorship associated with age-related increases in inflammation?

Purpose: In a cross-sectional study, we hypothesized that breast cancer survivors would show higher levels of CRP as compared to age-matched women who had no cancer history, and that this difference would be greater in older breast cancer survivors. *Methods and Results:* Breast cancer survivors (N=166) and comparison women (N=138) between the ages of 55 and 85 years were recruited concurrently, with assay of CRP. Breast cancer survivors show a greater rate of increase in CRP per year (lnCRP increase per year =0.055; P<0.01) as compared to comparison women (lnCRP increase per year = 0.037 mg/L; P>0.40), and cross the threshold of high risk CRP (>3 mg/L) 12.0 years earlier than the comparison group (71.3 vs. 83.3 years), with

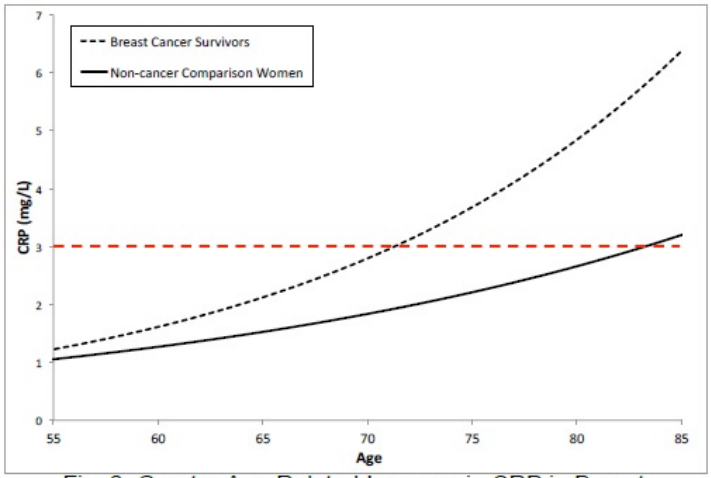


Fig. 2. Greater Age-Related Increase in CRP in Breast Cancer Survivors vs. Non-Cancer Comparison Women

adjustment for sociodemographic variables, body mass index (BMI) and use of anti-inflammatory medications (Fig 2). Cancer related variables (i.e., time since cancer diagnosis and treatment) did not alter the rate of change in the breast cancer survivors. IL-6 and sTNFrII showed a similar pattern *Conclusions:* These cross-sectional data provide a compelling rationale for the present study that will prospectively test whether breast cancer survivors show an accelerated rate of increase of inflammation as compared to an age-matched comparison cohort. Moreover, the annual rate of increase of inflammation is significantly greater in breast cancer survivors vs. comparison women, which indicates that 32 months longitudinal follow-up study is more than adequate to detect differences as a function of breast cancer status. Finally, it is not known what factors account for accelerated rate of inflammation, although our preliminary data suggest that sleep disturbance and/or depression history may independently contribute or interact with breast cancer status to accelerate increase in inflammation.[69]

C.1.2. Is the increase in inflammation in breast cancer survivors due to cancer-related treatments? *Purpose:* Chemotherapy may induce acute increases in IL-6, not other cytokines in cancer patients[70,71] This longitudinal study examined effects of adjuvant chemotherapy on inflammation within one year of treatment. *Methods and Results:* Breast cancer survivors who did (n=49) and did not receive chemotherapy (n=44) were compared 3 months after primary treatment, and then one year later. Controlling for age, BMI, and radiation treatment, IL-1ra, IL-6, and CRP did not differ between groups and did not change over time (all P>0.3).[72-74] *Conclusions:* During the early survivorship period, cancer-related treatments have limited impact on inflammation in the long-term survivorship period. Although this finding needs to be replicated in a longer and larger study, these data raise the possibility that the late-effects of treatments on inflammation might be due to other moderating or vulnerability factors such as sleep disturbance and depression history, as hypothesized.

C.1.3. Is breast cancer survivorship associated with shorter leukocyte telomere length? *Purpose:* Short telomere length is associated with breast cancer incidence[31,75-79] and predictive of mortality in breast cancer survivors.[10] This study evaluated telomere length between older adult breast cancer survivors vs. older adult women. *Methods and Results:* Pairs of breast cancer survivors (n=17; 67.8 ± 5.2 years; time since treatment 6.3 years) and older adult comparison women (n=17; mean age 68.5 ± 5.0 years) were matched by age, BMI and education. PBMC telomere length was shorter in breast cancer survivors as compared to comparison women (t=2.13 (1,16); P<0.05; Fig 3) *Conclusions:* Older adults breast cancer survivors have shorter telomeres, in addition to increases in inflammation, as compared to older women with no cancer history.

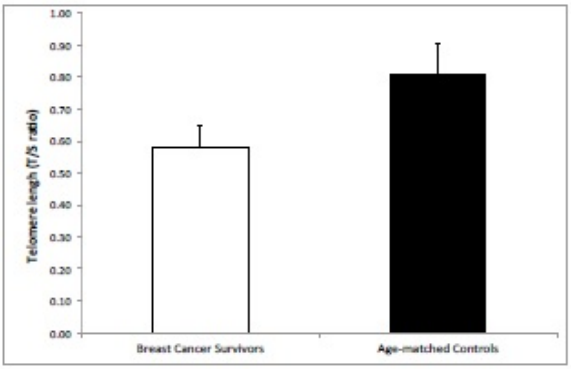


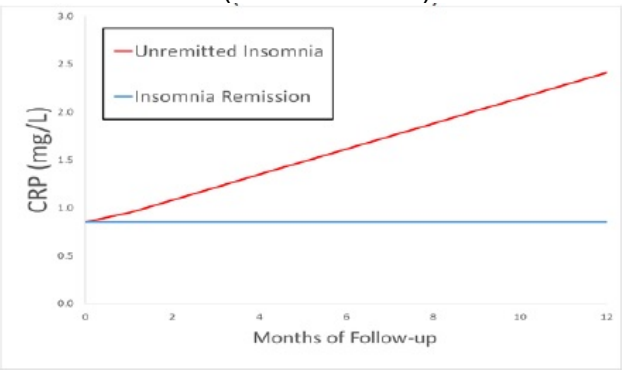
Fig. 3. PBMC Telomere Length Shortening in Breast Cancer Survivors vs. Matched Controls

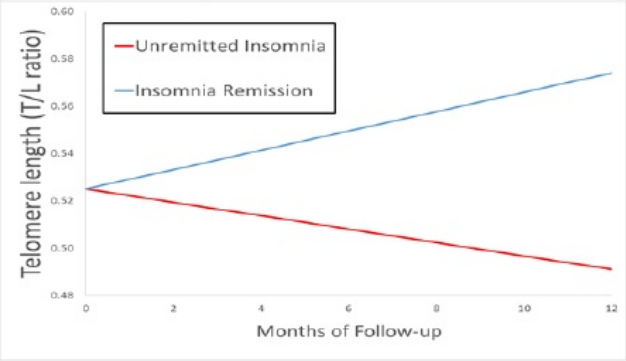
C.1.4. Does sleep disturbance drive inflammation?

Our systematic review and meta-analysis of sleep disturbance and inflammation in 72 cohort studies (N>50,000) has found that sleep disturbance is associated with higher levels of CRP (ES .12; 95% CI 5 .05-.19) and IL-6 (ES .20; 95% CI 5 .08-.31).[80] Our experimental research has further evaluated the role of sleep loss on inflammation,[11] and found that significant elevations in inflammation occur after only one night of sleep loss.[81] Additional studies examined the functional basis for altered inflammatory response after sleep loss, and found that sleep loss induces increases in spontaneous- and Toll-like receptor 4 (TLR4) stimulated monocytic production of proinflammatory cytokines;[14,16,82] increases in the activation of the nuclear factor (NF)- κB transcription control pathway[15] and signal transducer and activator of transcription (STAT) family proteins;[83] increases the transcription of IL-6 mRNA and TNF-α mRNA;[14] and increases in expression of the proinflammatory transcriptome.[14] Hence, given the impact of sleep disturbance on multiple levels of analysis of inflammation, this study will provide an assessment of both molecular and cellular markers of inflammation.

C.1.5. Does unremitted insomnia lead to increased rate of inflammation and telomere erosion? *Purpose:* To determine whether unremitted insomnia is associated with accelerated increases of inflammation and/or telomere attrition, and whether differences can be detected over 16 months. *Methods and Results:* In a 16 month randomized comparative efficacy trial of cognitive behavioral therapy for insomnia (CBT-I), Tai Chi Chih (TCC), and sleep seminar education control (SS) of 123 older adults with chronic insomnia, unremitted insomnia led to increased likelihood of high risk CRP levels (>3.0 mg/L) at 16 months (odds ratio [OR]=2.3;P < 0.05) and higher average levels of CRP (P<0.05) vs. insomnia remission.[50] In addition, unremitted insomnia

showed a greater annual rate of increase of lnCRP (0.51 mg/Ll) vs. insomnia remission (0.001 mg/L, P<0.05). *Also, unremitted insomnia shows an annual rate of increase of CRP that is over nine times greater than the rate in breast cancer survivors* (C.1.1). Similarly, unremitted insomnia is associated with greater annual rate of telomere attrition (0.034 T/L ratio) vs. those with insomnia remission (P<0.05).





Conclusions: Unremitted insomnia in the context of a randomized controlled trial led to increases the rate of inflammation and increases in the rate of telomere erosion, and these effects of sleep disturbance on the rate of change of biological aging are identified over 16 months, half the duration of this study. Given the effect sizes for the annual rate of change of CRP (0.97) and of telomere attrition (1.03), it estimated that n=18 and n=16 subjects per comparison group are needed to detect significant differences in inflammation and telomere erosion, respectively, which together support the feasibility of the sample size and duration of follow-up in this study. If sleep disturbance is found to have an independent effect on biological aging that is greater than breast cancer survivorship status, or is found to interact with breast cancer status, those with insomnia in both breast cancer- as well as non-cancer groups would be prioritized for insomnia treatments to reduce biological aging.

C.1.6. Is insomnia associated with greater age-related shortening of telomere length?

Purpose: Poor sleep quality and short sleep duration are associated with shorter telomere length, but no study has evaluated the impact of DSM-5 diagnoses of insomnia on age-related differences in telomere length. *Methods and Results:* In 106 older adults (71.4 ± 7.5 years; AG AG034588), stratified into two groups (DSM-5 insomnia history, n=36; no insomnia history, n=70), older age was significantly associated with shortening of telomere length in those with insomnia history (B=-.02, P=0.02; Fig 4), but not in those without insomnia history (B=-.002, P=0.78) although the correlation was similar to that found between age and telomere length in large epidemiological samples (i.e., B=-.005[84,85]) *Conclusions:* Among those with a history of insomnia, there is greater age-related telomere shortening, with an estimated 4 years of molecular aging for every 1 year of chronological aging in an average population, consistent with findings for depression.[62]

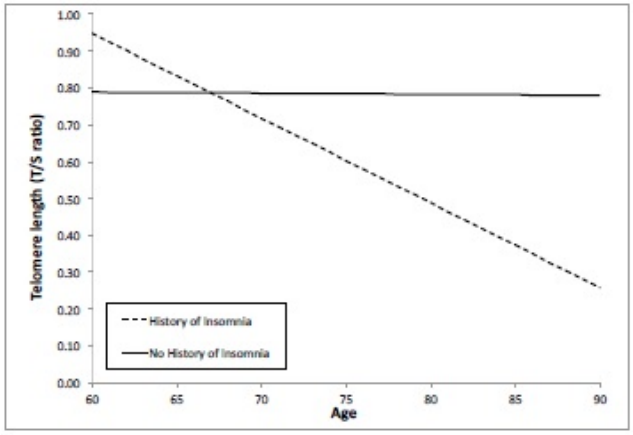


Fig. 4. Insomnia history predicts greater age-related shortening of PBMC telomere length in older adults

C.1.7. Does co-morbid sleep disturbance and depression history impact inflammatory activation?

Purpose: Whereas inflammation is known to be elevated in association with sleep disturbance and to be increased in the midst of a depressive episode, it is not known whether depression has an enduring impact on inflammation in those with sleep disturbance. *Methods and Results:* In a sample of older adults who had sleep disturbance (PSQI >5), those with depression history (n=125; 70.9 y) were compared those without a depression history (n=135; 71.2 y). Activation of NF-κB was increased in PBMC (t=3.6, P=0.06), monoctyes (t=5.4, P=0.02), and lymphocytes (t=3.7, P=0.05) in those with a history of depression and sleep disturbance. *Conclusions:* Co-morbid sleep disturbance and depression history induce an enduring increase inflammation.

C.1.8. Does co-morbid sleep disturbance and depression history impact telomere length?

Purpose: To evaluate whether sleep disturbance and depression interact to predict telomere length. *Methods and Results*: In a cross-sectional sample of 107 older adults (70.4 ± 7.7 y), an interaction between sleep disturbance and depression history was found (F=2.06 (3, 99) P=0.16; Fig 5); those with a sleep disturbance and depression history show shorter telomeres (n=19; 0.59±0.25 T/S ratio) as compared to those who had no sleep disturbance or depression history co- morbidity (n=86; 0.81±0.24T/S ratio; t(1,103)=2.22, P<0.05). Within women only, quintile analysis showed that none with co-morbid sleep disturbance and depression history were in the longest telomere length quintile, as compared to 20.8% with no co-morbidity (χ^2= 6.11, P=0.01). *Conclusions*: Sleep disturbance in older adult women with a history of depression is associated with shorter telomeres.

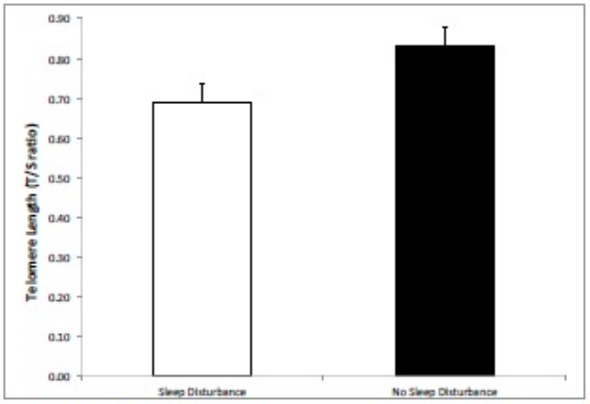


Fig. 5. PBMC Telomere Length Shortening in Older Women with Co-morbid Sleep Disturbance and Depression History

C.1.4. Summary

These cross-sectional and experimental studies show changes in cellular, transcriptional, and genomic markers of inflammation and telomere erosion between breast cancer survivors and a matched comparison group. *However, it is unknown whether breast cancer survivorship status is prospectively associated with accelerated age-related increases of inflammation and telomere erosion as compared to a comparison cohort*. Preliminary data also suggest that biobehavioral factors contribute changes in these markers of biological aging. Sleep disturbance is associated with increases in inflammation and activation of inflammatory signaling, as well as accelerated age-related decrease in telomere length in cross-sectional comparisons. Aside from our observations within the context of a controlled trial, *no prospective research has examined the contribution of sleep disturbance to increases of inflammation and telomere shortening, and whether sleep disturbance differentially accelerates inflammation and cellular aging in breast cancer survivors or in older adult women*. Finally, we have found that sleep disturbance and depression together contribute to greater increases in inflammation as well as telomere shortening*. The proposed study will provide novel understanding of the prospective roles of sleep disturbance, depression history, and their co-morbidity in accelerating the rate of biological aging in breast cancer survivors vs. a age matched comparison cohort***.**

C.2. RESEARCH DESIGN AND METHODS

C.2.1. Overview of setting, subjects, and procedures

To enable direct comparison of the breast cancer cohort and the matched comparison cohort (no cancer), the design and recruitment are identical to R01 CA160245, which is evaluating the prospective association between sleep disturbance, inflammation and depression risk in 300 breast cancer survivors who were identified through the KPSC SEER-affiliated tumor registry. This study will leverage this existing project, use the existing collaborative infrastructure with KPSC as the platform to newly recruit from the general KPSC membership. Along with the breast cancer survivors, we will prospectively follow this group of 300 matched comparison women who do not have cancer, but who have a similar prevalence of sleep disturbance and depression history. Inclusion of this comparison cohort in this prospective design is essential to address novel hypotheses of this study: 1) breast cancer survivors will show accelerated aging as compared to normal aging found in older adult women without a cancer history; 2) sleep disturbance will have a main effect and also interact with breast cancer survivorship to accelerate biological aging; 3) depression history will have a main effect and also interact with breast cancer survivorship to accelerate biological aging.

To recruit the older adult women without cancer from the KPSC membership, we will employ methods that will result in balanced study groups in terms of sleep disturbance and depression history and potential confounders, which minimize the effect of sequential rather than concurrent recruitment of the comparison group. First, both breast cancer survivors and older adult comparison women without cancer have equal access to care at KPSC, and will be recruited using identical methods from the KPSC membership. Second, given that disenrollment from KPSC is low, we will be able to retain individuals from both groups for the study's duration (over 80% of breast cancer patients remain enrolled over a 20-

years).[86] Indeed, during recruitment of the KPSC breast cancer survivors over the last two years in R01 CA160245, age, ethnicity, co-morbidity, and frequencies of sleep disturbance and depression history have not changed. Third, comparison subjects will be individually matched (1:1) to the breast cancer cohort based on the following characteristics that are strongly associated and hence propensity for sleep disturbance and depression history.[11,20,87] (see Section C.2.2).

Breast cancer survivors (55 to 85 years) and older adult women without cancer will be prospectively followed for 32 months (i.e., baseline, 8, 16, 24, 32 months). This companion study will extend the duration of follow-up from 24- (as proposed in CA160245) to 32 months for both breast cancer survivors and comparison women without cancer. The duration of the follow-up, lasting 32 months, was determined in part by our preliminary data, in which we found that the annual rate of change of inflammation (i.e., CRP, IL-6) is significantly greater in breast cancer survivors vs. comparison women. A period of 32 months follow-up is adequate to show change in inflammation and telomere attrition, as we also have found in a randomized controlled trial of insomnia treatment that a period of 16 months is adequate to show that unremitted insomnia leads to greater increases in CRP and decreases in telomere length as compared insomnia remission. Likewise a period of 3 months was adequate to detect significant increases in TNF and IL-1RA, as well as increases in NF-κB, in breast cancer survivors[88] or stressed older adult[89] who were participating in a randomized trial and receiving the control condition. Finally, one year was adequate to show a significant increase of IL-6 in a prospective study of 119 stressed older adults as compared to controls.[69] Follow-up over 32 months is also adequate to detect differences in the rate of change of telomere attrition between breast cancer survivors and comparison women, based on the rate of telomere attrition in breast cancer survivors participating in a randomized controlled trial and assigned to the control condition.[90] For telomere attrition, a period of 30 months was adequate in linking rate telomere erosion and mortality risk in breast cancer survivors,[10] and a one year follow-up period was adequate to detect greater rates of telomere attrition in relation to life stress in 239 healthy post-menopausal women.[91] The frequency of assessments every 8 months, rather than 12 months, provides additional statistical power to evaluate the proposed relationships; given subject burden and budgetary issues related to funding of CA160245, 6 month follow-up was not feasible. Repeated evaluation of sleep disturbance, as well as depression, will be obtained to test whether these behavioral mechanisms differentially moderate changes in the temporal profile biological aging.

Setting: Kaiser Permanente Southern California (KPSC) provides an ideal environment for population- based epidemiologic and clinical and health services research because of its diverse membership representation of residents in southern California, integrated model of care delivery, and electronic health records with encounter and outcome information, covering approximately 15% of the population in a seven- county area in southern California. Indeed, this study can possibly only be done using a large health care system such as KPSC, given the needed sample sizes, prevalence of breast cancer, sleep disturbance and history of depression, and availability of extensive automated clinical data to identify eligible comparison women based on the matching characteristics.

C.2.2. Subjects and Matching Criteria

Overview: This study leverages R01 CA160245 that has recruited 315 breast cancer survivors aged 55-85 years (above target N=300). Here we plan to enroll a comparison group of older women without cancer history from the general KPSC membership, who will be matched (1:1 matching) to the breast cancer cohort based on the following characteristics:[92] age at study entry; year of the breast cancer case's diagnosis (2007 through 2012) in which comparison woman must have also been a health plan member at least one year prior to the case's cancer diagnosis date; race/ethnicity; BMI, and Cumulative Illness Rating Scale-Geriatric (CIRS-G) score.[93] In CA160245, 39% of the breast cancer survivors have sleep disturbance and 35% have depression history. Hence, the comparison group will be identified via EHR and interview to ensure similar prevalence of sleep disturbance and depression history. Balancing group prevalence of sleep disturbance and depression will allow for testing of differences in biological aging between breast cancer survivors and comparison women without the confounding influence of "naturalistically" recruiting the two groups which would result in over 2-fold greater prevalence of sleep disturbance and depression history in the breast cancer survivors. EHR will identify comparison women with sleep disturbance and depression history who can be matched to the breast cancer survivors, with confirmation by interview.

As with the breast cancer cohort, the comparison cohort will be stratified by history of depression

to evaluate the relationships between sleep disturbance and depression history on rates of changes of inflammation and telomere biology. Eligible comparison women will include those with a lifetime major depression history as ascertained by the SCID for DSM-5.

Sample generalizability: KPSC members comprise nearly 1 in 16 residents of the Los Angeles area; the sample is ethnically- and sociodemographically representative of the general southern California population.[94]

Eligibility criteria**:** ***Inclusion criteria****:* The comparison cohort will fulfill the identical eligibility requirements of the breast cancer survivors who are enrolled as part of CA160245 with the following difference: the comparisons will not have a history of cancer at the time of study entry. The comparison cohort will be between the ages of 55 to 85 years of age and must have been enrolled at KPSC at least 12 months before study recruitment, identical to the breast cancer survivors. Inclusion of only postmenopausal women will reduce variability in inflammatory and sleep measures. ***Exclusion criteria****:* We will exclude women with the following sleep, psychiatric, and medical disorders:[95] (a) History of a sleep disorder other than insomnia (e.g., sleep apnea), as this study is evaluating the relationship between depression and insomnia sleep complaints; (b) Current psychiatric disorder (except chronic insomnia) including current major depression or alcohol or substance dependence as identified by the SCID for DSM-5, as the prospective association between sleep disturbance and depression occurrence, independent of other depressive symptoms, is being examined as part of CA160245. Subjects must be remitted of major depression, or other psychiatric disorder for 6 months prior to entry;[96,97] (c) Medication-related immune-suppression secondary to recurrent or other neoplastic disease, corticosteroids or other immunosuppressive therapy, given that such medication would confound the impact the effect of sleep disturbance on inflammatory biology; (d) Presence of significant underlying illness that would be expected to prevent completion of the study (e.g., dementia); (e) Any other condition (e.g. extensive psoriasis, chronic pain syndrome, cognitive impairment, severe hearing loss) that in the opinion of the investigator or treating physician might interfere with the evaluations and/or impact inflammatory biology; (f) Not ambulatory (bed-ridden); (g) Unable to commit to follow-up schedule; (h) No subject will be acutely suicidal or be considered a suicidal risk. We will include include women who are smokers, whose BMI is greater than 35 kg/m^2, or who are using non-steroidal anti-inflammatory medications,[98,99] (all of which might impact inflammation) to ensure broad generalizability of the findings. The statistical analyses will control for effects of these factors.

Sample availability for non-cancer comparison women:

We have sufficient pools of potential comparison women in the active KPSC membership, who will be randomly selected from these non-overlapping strata. To ensure ample eligible comparison women, we will identify up to 4 matched women (replacements) for each breast cancer survivor. We will over-enroll (N=360) in order to ensure a target comparison cohort of 300 KPSC women without a history of breast cancer. The comparison cohort will be followed for 32 months similar to the breast cancer cohort. As KPSC health insurance retention is very high (80% over 20 years), we do not anticipate substantial disenrollment. The comparison cohort will be initially linked with mortality, outpatient and inpatient records, and pharmacy and membership files to determine which women are eligible for recruitment.

C.2.3. Procedures

Eligibility and depression screening: The recruitment and screening process will include physician consent to approach KPSC members; pre-interview invitation letter; patient consent for phone screening; and screening for eligibility and interest in study participation. *Depression screening eligibility.* To determine screening eligibility for study entry, prior history of depression will be assessed by three screening questions: 1) Have you ever been depressed nearly every day for two weeks or more?; 2) Have you ever lost interest in normal activities nearly every day for two weeks or more?; or 3) Have you ever been prescribed an antidepressant medication?. These three questions are highly sensitive (>95%) in identifying those with a prior history of depression, which will be confirmed by SCID interview data obtained during baseline assessment.[95] Comparison women with current depression will be excluded, consistent with CA160245

Baseline and Follow-up assessments: In a face-to-face interview format lasting about 120 minutes, the measures listed in Table 1, will be obtained. Because infections alter inflammatory markers, subjects will be scheduled at least two weeks after such occurrence. To maintain high follow-up rates which is >95% in the CA16045, we provide financial incentives (up to $50 per interview), obtain contact

information for someone who does not live with the participant, send a letter confirming the visit, and give a telephone reminder.[100]

Table 1: List of measures that will be obtained, and the timing of assessment

	EHR*	Entry	8 mo	16 mo	24 mo	32 mo
Grouping and screening variables						
Breast- and other cancer history and status	X	X	X	X	X	X
Sleep / fatigue assessment						
Duke insomnia diagnosis		X	X	X	X	X
Pittsburgh Sleep Diary/Online Sleep Diary System		X	X	X	X	X
Pittsburgh Sleep Quality Index / Insomnia Severity Index		X	X	X	X	X
Actigraphy		X	X	X	X	X
Fatigue Symptom Inventory / Multidimensional FSI		X	X	X	X	X
Use of sleep medications		X	X	X	X	X
Depression assessment						
Depression screening PHQ-9**		X				
SCID-DSM-5 and depression treatment history		X	X	X	X	X
Beck Depression Inventory/ Beck Anxiety Inventory		X	X	X	X	X
Use of antidepressant medications		X	X	X	X	X
Inflammatory and cellular aging markers						
CRP, pro- and anti-inflammatory cytokines		X	X	X	X	X
Inflammatory transcriptional signaling (NF-κB)		X	X	X	X	X
Microarray (subsample N=100 cases/ 100 comparisons)		X		X		X
PBMC telomere length		X	X	X	X	X
Demographics and medical history						
Demographics		X				
Recent medical history (newly diagnosed conditions)	X	X	X	X	X	X
Biobehavioral confounds, psychotropic medications	X	X	X	X	X	X
Health status/quality of life						
Cumulative Illness Rating Scale-Geriatric: Frailty Index	X	X	X	X	X	X
SF 36; Clinical Syndrome of Frailty		X	X	X	X	X
Godin Leisure Physical Activity Survey; FitBit activity		X	X	X	X	X
PROMIS 29 Profile, Global health, Pain		X	X	X	X	X
Stress / social support assessment						
Perceived Stress Scale		X	X	X	X	X
Social Provisions Scale – Attachment		X	X	X	X	X
UCLA Loneliness Scale		X	X	X	X	X
Interpersonal Support Evaluation List		X	X	X	X	X

*Electronic Health Records (EHR) data on existing diagnoses and medical treatments will be collected from automated sources which avoid recall bias. **The PHQ-9 will be used to monitor depression including suicidality, with confirmation by SCID-V interview.

C.2.4. Clinical and Biomarker Measures

Primary outcome of biological aging: inflammation and telomere length

Inflammatory mechanisms: We will measure inflammatory biology dynamics using a vertically integrated mechanistic approach and examine gene expression, signaling pathways, and circulating levels based on our published studies.[11,50,101] KPSC members will have blood sampling at the UCLA CTSI between 9 and 11 a.m.; samples are processed within minutes after being obtained by the Cousins Center Inflammatory Biology Core, consistent with procedures of CA160245. *Circulating levels* will be assessed in plasma collected at 4°C from whole blood samples, frozen at -80°C, and stored at the UCLA Cousins Center. Inflammatory and anti- inflammatory markers will be assayed using a multiplex panel of inflammatory (IL1-β, IL-6, IL-8, TNFα, IL-10) (R&D Systems Luminex Performance Human High Sensitivity Cytokine Panel); CRP will be determined by high sensitivity ELISA.. The R&D multiplex assay shows acceptable reproducibility (inter- and intra-assay variability <16%)[102] and strong correlations (r>0.90) with R&D high sensitivity ELISAs.[103] All samples from each woman across the study period will be assayed at the same time in duplicate determinations; assay variability will be monitored by the inclusion of an internal laboratory quality control sample on every assay. **All sample assays will be performed at the end of the study, with concurrent assays of samples from both groups**. *Inflammatory signaling* will be measured by assay of activation of NF-κB in nuclear extracts by ELISA.[89] *Microarray-based genome-wide transcriptional analyses* will be used identify the molecular inflammation- related signaling pathways that are plausible candidates for molecular mediators of inflammation (e.g., NF-κB), and glucocorticoid receptor-related signaling pathways that may underlie increased inflammatory signaling in association

with sleep disturbance or depression in a subsample (N=100 breast cancer survivors/100 comparison women), using methods previously described.[14] In addition, given our preliminary findings that sleep loss enhances Proinflammatory Senescence Associated Secretory Phenotype (SASP) and increases cellular senescence, a priori selection of genes representative of key components of the predominantly inflammatory response in senescent cells will be examined: *IL6, CSF2, CCL8, IL8, CCL13, ICAM1, CXCL1, CXCL2, CXCL3* and signals known to be upregulated during cellular aging, the DNA damage response (DDR) *GADD45GIP1, TP53BP1, CHEK1, TP53, TERF2, SIRT1, TERT, GADD45A*. Bioinformatic analyses of transcriptome dynamics will be performed as previously described.[101,104]

Telomere length: Telomere length will be determined using a real time quantitative polymerase chain reaction (qPCR) methodology as described previously,[105-108] with reliability testing in collaboration with Dr. Rita Effros (UCLA). Peripheral blood mononuclear cells (PBMC) are isolated and genomic DNA is extracted. Using the standard curve method, cycle threshold (CT) values are then plotted on a standard curve of human genomic DNA to estimate ng/microliter concentration values. Telomere length values are expressed as the ratio of the estimated concentration generated by PCR of the telomere gene (T) divided by the hemoglobin single (S) copy gene = (T/S). Samples are run in triplicate and are then assessed for reliability and mean values are then calculated. This PCR methodology has been reliably performed across numerous studies at UCLA Inflammatory Biology Core Laboratory and in the laboratory of Dr. Effros with low intra-assay and inter- assay variability, consistently below 5%. Validity of this methodology has been confirmed by comparing relative T/S values with absolute values derived from Southern blot methods by ourselves (r^2 = .94) and others (r^2 = .68).[106] Isolated PBMC will be used to determine telomere length, because granulocytes are a potential source of telomere length variability; 50-80% of leukocytes are granulocytes and this cell type with longer TL shows a reduced rate of attrition with age.[109,110] In addition, a complete blood count (CBC) will determine cell distribution in our PBMC samples, and flow cytometric analyses will be conducted in a subset of subjects to determine whether change in PBMC telomere length is due to shifts in cell subsets (e.g., B, NK, T helper, cytotoxic T, and monocyte cells), including percent of late differentiated/senescence cells (e.g., CD4+/CD8+CD28-CD57+).[111]

Insomnia and sleep disturbance assessment: Sleep will be evaluated on a categorical and dimensional basis. The primary grouping variable will be PSQI>5 (98% sensitivity; 84% specificity for insomnia).[112] as the aim of this prospective study is to guide the development of a prevention trial. If sleep disturbance is found to be a significant risk factor for inflammation and telomere attrition in breast cancer survivors and/or comparison women, then a simple questionnaire approach (i.e., administration of the PSQI) could be utilized to target those at greatest risk for entry into a prevention trial to improve sleep and reduce rates of biological aging. We are using the PSQI, rather than the Insomnia Severity Index (ISI) because we have found that the PSQI predicts increases in inflammation, telomere attrition, and depression. This approach will be supplemented by use of the Structured Clinical Interview for DSM-5 and the Structured Clinical Interview for Sleep Disorders to evaluate specific sleep disorders according to DSM-5[113] and ICSD-2[114] criteria. Lifetime history of insomnia will be assessed given findings that the profile of inflammatory activation may be related to duration of insomnia.[11] The *Insomnia Severity Index (ISI) (*86% sensitivity; 88% specificity for insomnia) will also be used.[112] Sleep quality will be assessed by the *Pittsburgh Sleep Quality Index (PSQI).*[115,116] For two weeks prior to the interview, participants will complete a *Online Sleep Diary System* with daily assessment of sleep and wake times, accessible via computer interface with unique user IDs. This self- report data will be coupled with *wrist actigraphy* (Motion Logger with light) *as* an objective assessment of sleep behaviors and sleep duration. Exclusion for sleep apnea will use *Berlin Questionnaire for Sleep Apnea)*[117] as well as objective assessment with the FDA- approved *WatchPAT device*. Daytime dysfunction will be evaluated by assessment of fatigue using the *Fatigue Symptom Inventory (FSI)* and the *Multidimensional Fatigue Symptom Inventory-Short Form (MFSI-SF).*[118-120] Finally, we will obtain history of sleep medication use from the KPSC EHR, and confirm use by interview data, as well as questionnaire (i.e., PSQI).

Depression history assessment: At baseline and each follow-up assessment, the *Structured Clinical Interview for DSM-5 (SCID-5)* will be used to diagnose a lifetime history of major depressive disorder,[121] as well as to ascertain relevant clinical data about number of episodes, age of onset, last episode, and treatment. We have over two decades of experience in the administration of the SCID and training staff to achieve criterion validity using standardized SCID training materials. Because depressive symptoms might co-occur with sleep disturbance, and confound interpretation of the hypothesized

relationships between sleep disturbance and biological aging, *Beck Depression Inventories* will be covaried in the analyses.[122,123]

Biobehavioral and medical factors that might be associated with inflammation and telomere length as noted in Fig 1 (Table 1), include *demographic information* such as age, gender, race/ethnicity, education, household income, current and previous occupation, marital status, and household composition; *biobehavioral confounds of inflammation*[98] such as alcohol consumption, smoking history, BMI, and physical activity (i.e., *Godin Leisure-Time Exercise Questionnaire;* to provide an objective assessment that can be correlated with the self- report questionnaires, Fitbit will be used in a random sample of 150 breast cancer survivors and 150 matched comparison women);[124] medications including sleep medications, antidepressant medication, statins and non- steroidal anti-inflammatory agents, and recent infections in last week; and *medical co-morbidity* (i.e., *CIRS- G*[93]); Frailty Index by EHR[125]; health functioning (ie., *Medical Outcomes Study Short-form SF-36**[126-128]) and assessment of the Clinical Syndrome of Frailty;[125,129] *Insomnia and depression treatments* (i.e., use of antidepressant- and sleep medication) will be characterized at baseline and follow-up.[95]

Psychosocial factors will be assessed including number and perceived threat of recent life stresses (i.e., *Perceived Stress Scale*)[130] and subjective social isolation that is associated with depression, sleep disturbance and inflammation (i.e., *10-item Revised UCLA Loneliness Scale*,[131] *Interpersonal Support Evaluation List.*[132]

C.2.5. Statistical Analyses

Overview of the analytic plan: Breast cancer survivors and the comparison cohort will be examined for baseline equivalency using univariate and bivariate statistics. Data will be carefully reviewed for distributional assumptions and corrections made as needed. The amount of missing data is expected to be minimal; yet, analytic strategies will be selected so that missing data will not eliminate a given case (e.g., mixed models ANOVA). We will examine the set of sleep predictors (i.e., PSQI, ISI, diagnosis, actigraphy) to assess validity and reliability of sleep disturbance. Depression history status will be determined using SCID-5. Given the matching in the design, we will conduct a matched analysis (e.g, conditional multivariable logistic regression).

Testing of the primary hypotheses will follow a straight-forward approach: overall pattern and linear growth curves for the inflammatory and telomere length outcomes over the 32 month prospective follow-up period will be compared between the relevant groups (baseline, and four follow-up measures spaced 8 months apart). Based on the study design, the comparison cohort will be specifically balanced on sleep disturbance and depression prevalence history given the distribution in the breast cancer cohort (39% with sleep disturbance and 35% with depression history). Thus the general analytical plan is mixed models ANOVA with repeated measures and three two-group variables: breast cancer, sleep disturbance, and depression history. The hypotheses are tested within the ANOVA.

Hypothesis 1: The effect of interest is the main effect of breast cancer survivorship.

Hypothesis 2: This focuses upon the main effect of sleep disturbance (PSQI >5) and the 2-way interaction of sleep disturbance with breast cancer survivorship. Further exploratory analyses (noted below) will consider sleep disturbance as varying by time over the follow-up period.

Hypothesis 3: The two effects are main effect of depression history and the 2-way interaction of depression history with breast cancer survivorship. Further exploratory analyses will consider depression status as varying by time over the follow-up period.

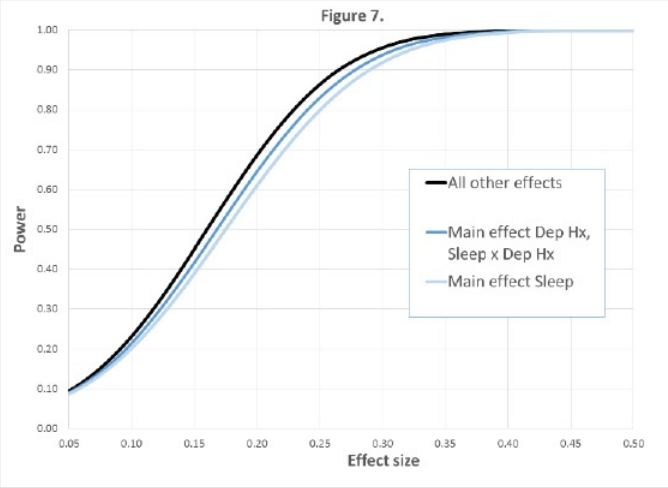
If there are main effects for sleep disturbance and/or depression history, then the hypothesis that these risk factors accelerate biologic aging will be confirmed. If there are interaction effects for these two risk factors with breast cancer survivorship, then the hypothesis will be confirmed that breast cancer survivors show accelerated biologic aging related to these risk factors.

Exploratory Hypotheses: The 2x2 interaction of sleep disturbance and depression history will be examined along with the 3-way interaction of 2x2x2 (breast cancer status, sleep disturbance, depression history) to elucidate potential synergistic effects of sleep disturbance and depression as moderators both across the breast cancer factor as well as in interaction with it.

Addressing potential confounding of inflammation and telomere length: As a first step is to reduce confounding, we will exclude patients with conditions that could complicate the assessment of inflammation and telomere biology (e.g., routine use of steroids) and to generate comparable groups on others (e.g., age, race/ethnicity). The second step is in the analysis stage with examination of potential confounding variables and determine if results hold when these variables are controlled. Potential

covariates (beyond the matching factors) include socioeconomic status, alcohol use, smoking status, use of medications with known effects of inflammatory markers (e.g., statins, regular use of NSAID, antidepressant medications), and physical activity.
As described in CA160245, separate modeling within the breast cancer group will test cancer and treatment related variables, including disease stage classification. Hence, some analyses will only include the breast cancer cohort. In such analyses, breast cancer treatment will categorized into three groups: surgery only (with or without radiation); surgery and chemotherapy; surgery, radiation and chemotherapy.
Addressing role on baseline factors: We will explore baseline factors (e.g. phenotypic frailty) associated with accelerated cellular aging in breast cancer survivors and comparison women without cancer.
Addressing potential baseline differences in biological age: Groups will be matched on chronological age. However it is also possible that the groups may differ on biological age at baseline (i.e., different baseline levels of inflammation and telomere length), as breast cancer survivors may already exhibit accelerated aging. Initially, baseline differences in biologic aging outcomes will be assessed; should the groups differ at baseline, the baseline values will be addressed in the planned analyses (which examine change over the 32 months follow-up) so that we can determine the prospective rate of biological aging taking into account entry levels. If it appears the change over time in biological age is not linear, appropriate transformations will be applied to linearize change given baseline values.
Addressing change in moderator status over time: As this is a prospective study, group status is fixed at baseline. Nonetheless, as exploratory analyses, changes in the predictor domains (i.e., sleep disturbance and depression) will be examined by mixed models repeated measures analysis of variance with time varying factors. New cases of depression and changes in sleep disturbance status will be recorded during the follow- up period and then modelled as predictor of change in biological age (inflammation and telomere length).
Statistical power considerations.

Sample sizes were based upon the three primary hypotheses being tested at least 80% power using a two- tailed tests at alpha = 0.05 to detect effects small to medium effect sizes under the assumption that effects smaller than this would not be clinically meaningful. As can be seen (Figure 7), effects of size 0.25 are detectable at 80% power with minimum total n=600 even given some imbalance in the groups from matching observed prevalence of sleep disturbance and depression history within the breast cancer group. Nonetheless, with this design there is somewhat more power for the 3-way interaction assuming a small effect (nominal d=0.25).



Many of the cross-sectional results shown above involve larger effect sizes (d=.55 to d=1.21) and some of our results suggest medium to large effects expected prospectively over 32 months. Depending on the pattern of change during the follow-up period, power may be improved from the use of repeated measures (especially if change is mostly linear or can be linearized). To summarize, the statistical power will be more than sufficient to properly test every main effect and interaction of interest within the primary and exploratory hypotheses assuming they are a substantial and clinically meaningful factor.
C.2.6. Time Frame Over the last two years of CA160245, we have surpassed our target of 300 breast cancer survivors (N=315) from the KPSC, with follow-up for 32 months ongoing. A similar timeline is proposed for this study, and we do not anticipate any difficulties identifying 300 matched KPSC older adult women without a cancer history.
C.2.7. Future Directions Findings from this study would provide a compelling rationale to examine the impact of accelerated biologic aging on risk of age-related chronic disease and phenotypic frailty in cancer survivors and controls, risk of cancer recurrence in the breast cancer survivors, and the predictive strength of modifiable risk factors such as sleep disturbance on these outcomes. To this end, we will use the EHR system of KPSC to track future outcomes in both populations, and test whether such risk is tied to markers of biological aging and whether this risk is greater in breast cancer survivors.[9,77] Findings from

this study would also support the rationale for testing strategies that target multiple pathways that drive inflammation. For example, interventions (i.e., exercise) that target other factors related to inflammation (i.e., physical activity, BMI) might be coupled with interventions that solely target behaviors (i.e., sleep disturbance) to augment the efficacy of prevention strategies to reverse biologic aging. Further, combining mind-body interventions that target stress effector mechanisms (i.e., SNS activity, HPA axis sensitivity) might add to the efficacy of aspirin or NSAIDS alone (i.e., blocking only COX-1/COX-2), especially since the effect sizes of mind-body interventions on inflammation appear to be as robust as the anti-inflammatory effects of aspirin or NSAIDS.[50,101,133]

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