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Does walking during chemotherapy impact *p16*^{INK4a} levels in women with early breast cancer

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Abstract

Background: Increased *p16*^{INK4a} (p16) expression is directly related to cellular senescence and is a robust biomarker of aging in humans. Prior studies have shown that levels of p16 dramatically increase in breast cancer patients who have received adjuvant chemotherapy. This study investigated whether moderate physical activity during chemotherapy would attenuate the expected rise in p16 expression.

Methods: Participants were women with Stage I–III breast cancer enrolled in a walking study for the duration of their chemotherapy (NCT02167932, NCT02328313, NCT03761706). Participants were asked to walk at least 30 min or 6200 steps/day following a structured walking program and to wear an activity tracker. p16 mRNA levels were measured in peripheral blood T-cells before chemotherapy initiation and at approximately 6 months after last chemotherapy treatment (mean 200 days, SD 40 days).

Results: In total, 141 participants met inclusion criteria and 10% ($n = 14$) averaged > 6200 steps/day. There was no significant association of daily steps with change in p16 levels pre- to post-chemotherapy (Pearson correlation coefficient = 0.11, $p = 0.17$). After adjusting for age, stage, anthracycline-based chemotherapy, and baseline p16, the change in $\log_2$ p16 for each 1000 steps was estimated to be 0.03 ($p = 0.35$). Most participants were sedentary prior to chemotherapy and achieved modest levels of physical activity during treatment.

Conclusion: A self-guided walking program achieved only modest levels of physical activity and was unable to ameliorate chemotherapy-induced change in p16 levels in women undergoing chemotherapy for early-stage breast cancer. More structured and vigorous exercise programs should be tested for a more definitive exploration of their impact on post-chemotherapy p16 levels.

KEYWORDS
adjuvant chemotherapy, breast cancer, exercise, intervention, p16, walking

1 | INTRODUCTION

Aging is a heterogeneous process among humans, and chronological age is often an inconsistent predictor of health status.[1] Over the past decade, biomarkers have emerged as a novel method for measuring molecular age in pursuit of more accurate clinical assessments and personalized treatment plans. The $p16^{INK4a}$ (p16) gene encodes a protein that leads to cellular senescence and has been identified as a critical correlate of cell-intrinsic, molecular aging in humans.[2–5] Senescence is a cellular mechanism that promotes permanent inhibition of the cell cycle in response to cellular stress or DNA damage. Over time, the accumulation of senescent cells in tissue reduces replicative function and promotes inflammation.[6,7] The expression of p16 mRNA in peripheral blood T-lymphocytes (PBTL) is an accurate indicator of this molecular aging process.[8]

The expression of p16 has been studied in the context of multiple disease processes,[9,10] including breast cancer and chemotherapy treatments.[1,11] P16 RNA expression dramatically increases in early-stage breast cancer patients following the initiation of (neo)adjuvant chemotherapy, especially in patients receiving anthracycline-based regimens.[12] Anthracycline-based chemotherapies have demonstrated accelerated molecular aging by 23–26 years, compared with non-anthracycline-based regimens, which accelerated molecular aging by 9–11 years.[11] Baseline (pre-chemotherapy) p16 levels, but not chronological age or race, are also associated with the magnitude of increases in p16 levels after chemotherapy, specifically patients with lower p16 levels at baseline are more likely to experience larger increases in p16 levels post chemotherapy treatment.[11]

p16 expression has also been studied in the context of lifestyle choices. Higher p16 levels positively correlate with physical inactivity and tobacco consumption.[8] These findings led us to hypothesize that physical activity during chemotherapy for early breast cancer might mitigate the rise in p16 levels observed in our previous research. Current Center for Disease Control and Prevention (CDC) and American Cancer Society guidelines recommend 150 min of physical activity per week for adults with cancer, with guidance from the treating oncologist for patients in active treatment such as chemotherapy.[13–16] As in adults without a cancer diagnosis, the majority of adults with cancer generally do not meet guideline-recommended levels of physical activity.[17–21] For the current study, our primary aim was to evaluate whether adherence to a self-directed physical activity intervention throughout chemotherapy treatment would be associated with smaller pre-post chemotherapy rises in p16 levels compared with patients with lower physical activity adherence.

2 | METHODS

2.1 | Participants

Our sample was drawn from participants in three studies that included similar eligibility criteria and the same physical activity intervention — NCT02167932 (age 21–64), NCT02328313 (age 65 and older), NCT03761706 (age 22 and older). Patients provided written or electronic informed consent meeting all federal, state, and institutional guidelines, and the studies were approved by the University of North Carolina Lineberger Comprehensive Cancer Center Protocol Review Committee and the Institutional Review Boards for each study site (4). Descriptions of these studies have been previously published.[22,23] Eligibility criteria were: female, breast cancer diagnosis (Stage I, II or III), and scheduled for adjuvant or neoadjuvant chemotherapy. Patients with any form of prior chemotherapy were excluded. All participants had to have p16 values assessed pre-chemotherapy (baseline) and post-chemotherapy.

2.2 | Intervention

The home-based walking intervention was based on an adaptation of the Arthritis Foundation's evidence-based Walk With Ease (WWE) program.[16,24] Specifically, the intervention consisted of walking guideline-recommended 150 min/week (CDC and American Cancer Society guidelines) at a pace that was safe, comfortable, and sustainable for the duration of chemotherapy. We have previously described how we estimated that achievement of 150 min/week at a moderate pace amounted to approximately 6200 steps/day.[16,25] Participants received an activity tracker (Fitbit™ or GARMIN™) and were asked to wear the tracker throughout their waking hours. Every 2–3 weeks, during a scheduled chemotherapy visit, research assistants uploaded activity tracker steps directly into research computers. Research assistants also asked participants about their walking and strength training, while providing positive encouragement and reassurance to those who were not meeting goals at each chemotherapy visit. Study participants were also given a copy of the WWE workbook, which includes chapters on overcoming barriers to walking, developing a walking routine, and other resources for staying motivated.[26]

2.3 | p16 expression

Blood samples were collected from study participants within 1 week prior to chemotherapy initiation and at approximately 6 months after their last chemotherapy treatment (mean 200 days, SD 40 days, range 105–336 days). Blood was drawn into lavender top EDTA tubes, T cells were isolated, and expression of p16 mRNA in peripheral blood T lymphocytes was determined using TaqMan real-time quantitative reverse transcription polymerase chain reaction. Expression analysis was performed by Sapere Bio (Research Triangle), using technology described previously.[12] Every assay included external and internal controls to monitor assay performance. Cycle threshold values over 37 were considered below detection and excluded from the analysis. The same assay and quality control procedures were used in every run. P16 is reported in log base 2 units.

TABLE 1 Pretreatment patient characteristics

Variable	Overall N = 141
Age, year	56.9 (SD, 13)
Race	
White	106 (75%)
Black	24 (17%)
Other	11 (8%)
Comorbidities	132 (94%)
Arthritis/Rheumatism	42 (31%)
High blood pressure	45 (33%)
Heart disease	6 (4%)
Diabetes	9 (7%)
Stroke	2 (2%)
Marital status	
Single, never married	22 (16%)
Married	78 (55%)
Separated, divorced, or widowed	41 (29%)
Live alone	32 (23%)
BMI	
<18.5 (underweight)	2 (1%)
18.5–25 (normal)	42 (30%)
25–30 (overweight)	43 (31%)
30+ (obese)	54 (38%)
Breast cancer stage	
I	29 (20%)
II	74 (53%)
III	38 (27%)
Hormone receptor status	
Negative	60 (43%)
Positive	81 (57%)
HER 2	
Negative	100 (71%)
Positive	41 (29%)
Triple negative	40 (28%)
Surgery	
None	3 (2%)
Lumpectomy	63 (45%)
Mastectomy	75 (53%)
Therapy	
Radiation	102 (72%)
Endocrine	68 (50%)
Chemotherapy timing	
Neoadjuvant	58 (41%)
Adjuvant	83 (59%)
Chemotherapy regimen	
AC-T (Doxorubicin/Cyclophosphamide plus Paclitaxel)	45 (32%)
AC-TC (Doxorubicin/Cyclophosphamide plus Paclitaxel/Carboplatin)	16 (11%)

TABLE 1 (Continued)

Variable	Overall N = 141
TC (Docetaxel/Cyclophosphamide +/− HER-2)	34 (24%)
TCH (Docetaxel/Carboplatin/anti-HER-2)	29 (21%)
Other	17 (12%)
Chemotherapy containing anthracycline	62 (44%)
Frequency of vigorous physical activity[a]	
Never	36 (26%)
A few times per month	28 (21%)
1–2 times per month	27 (20%)
3–4 times per month	33 (24%)
5 or more times/week	12 (9%)
Smoking history	
Never smoked	84 (60%)
Used to smoke	42 (30%)
Currently smoke	15 (10%)
Alcohol consumer	
Yes	51 (37%)
No	52 (37%)
Almost never	36 (26%)

[a]Defined as at least 10 min of physical activity that causes heavy sweating or large increases in heart rate or breathing.

2.4 | Statistical analysis

Descriptive statistics are provided to summarize the study sample. Associations with the primary outcome of interest (change in $\log_2$ p16) were evaluated using Pearson correlation coefficients and linear regression modeling. Changes in $\log_2$ p16 were calculated and used as the outcome measure for analysis.[12] Linear regression requires a normality assumption; based on a histogram of the data, QQ plot for quantiles, and the Kolmogorov–Smirnov test for normality, it was concluded that using linear regression was appropriate for the outcome measure of change in $\log_2$ p16. Unadjusted and adjusted (for age, anthracycline, and baseline p16) modeling was performed. All analyses were conducted using SAS statistical software v9.4. All statistical tests were two-sided, and a p value < 0.05 was considered statistically significant.

3 | RESULTS

3.1 | Patient characteristics

Table 1 shows the baseline pre-chemotherapy characteristics of the 141 study participants. The mean age was 57 years (range 28–83 years), and the majority of participants were white. Almost 94% of participants had comorbidities, with arthritis and high blood pressure being the most common. Over half of participants had a body
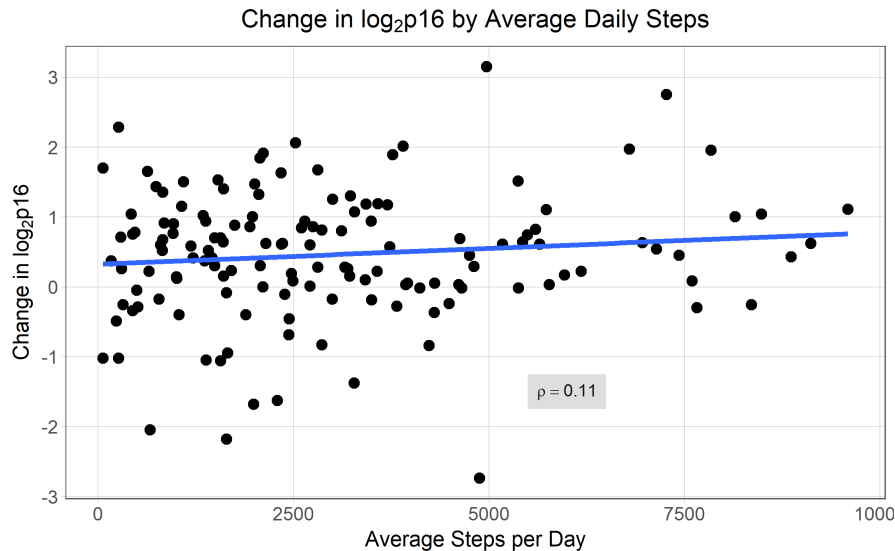
Change in log$_2$p16 by Average Daily Steps

$\rho = 0.11$

Change in log$_2$p16 by Pre-Treatment log$_2$p16

$\rho = -0.45$
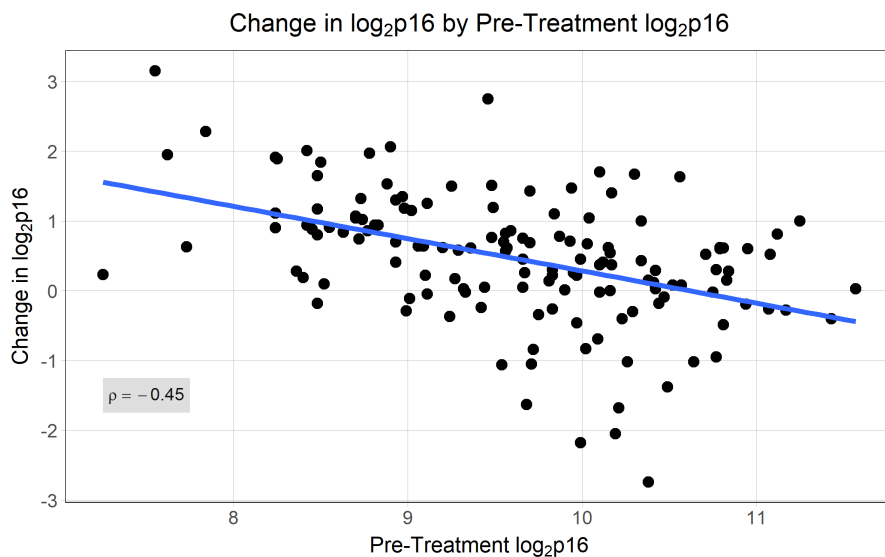
mass index/BMI > 25, and only 32% reported engaging in vigorous physical activity on a weekly basis pre-chemotherapy. A majority of participants had a Stage II breast cancer diagnosis, with 28% having triple negative tumors. Roughly 41% of participants received neoad-juvant chemotherapy and 59% adjuvant chemotherapy, with 44% of regimens containing anthracycline.

3.2 | Physical activity associations with change in p16

The average baseline log$_2$ p16 was 9.62 (SD 0.89, 95% CI: 9.47–9.77). During chemotherapy treatment, the average steps/day was 3025 (95% CI: 2651–3398 steps/day). Only 14 participants were able to average ≥ 6200 steps/day (corresponding to 150 min/week of walk-ing at a moderate pace). There was no association between average daily activity tracker steps and change in p16 (Pearson correlation coefficient = 0.11, $p = 0.17$, Figure 1). For each 1000 step increase in average steps/day, the estimated change in log$_2$ p16 was increased

0.047. This non-significant finding did not change after adjustment for age, stage, anthracycline chemotherapy, baseline log$_2$ p16. In a model adjusted for age, stage, anthracycline chemotherapy, and baseline p16, the estimated increase in log$_2$ p16 change for each 1000 steps was reduced to 0.03 ($p = 0.35$).

For the entire cohort, the average change in log$_2$ p16 was 0.46 (SD 0.92; 95% CI: 0.30–0.61), which is similar to previous findings for women treated with adjuvant chemotherapy.[11] Baseline p16 was a strong predictor of change over time, with a Pearson correlation coefficient of −0.45, $p < 0.0001$. Higher p16 at baseline was asso-ciated with less change in p16 over time (Figure 2), consistent with previous findings.[11]

4 | DISCUSSION

Although we hypothesized that increased physical activity lev-els would slow the dramatic increase in p16 expression follow-ing chemotherapy treatment, that relationship was not observed

in our cohort. It is important to note that only 9% of participants had engaged in vigorous physical activity a few days a week prior to their participation in our study, and only 10% were able to meet their physical activity goal of 6200 steps/day during chemotherapy despite encouragement. This sedentary patient population prior to chemotherapy is representative of the general population of women diagnosed with breast cancer.[18,27,28] In addition, in a prior study by our group using an identical walking intervention for early breast cancer patients less than 65 years of age, only 19% of participants were fully adherent to the walking goal.[16] Said study further examined patient-specific factors associated with step count including BMI, education level, race, and baseline physical activity.[16] Factors such as race were not included in our analysis given the low number of non-white participants ($n = 35$). Activity tracker data have shown that during the first week of chemotherapy, only 33% of this patient population is walking 100 min/week.[29]

Our study underscores the challenges to implementing even a simple exercise regimen in breast cancer patients undergoing chemotherapy. We had hypothesized that a simple walking program meeting the activity criteria of the CDC and American Cancer Society would have ameliorated the increase p16 expression levels that we have found in up to 75% of women receiving adjuvant chemotherapy.[12]

Our study has some limitations. Frist, the intervention period was limited to the duration of chemotherapy, while the p16 "change" period was from pre-chemotherapy to approximately 6 months post chemotherapy completion. It is possible that a longer intervention period mirroring the p16 measurements would have shown greater impact from physical activity. Second, more intense or supervised encouragement of the walking exercise and the inclusion of weight training could be considered for future studies.[30,31]

In our present study, we were not able to achieve even modest walking activity during chemotherapy, and it remains uncertain if a more vigorous and personalized exercise intervention might ameliorate the p16 rise seen with chemotherapy. Despite these findings, exercise should still be encouraged in this patient population as walking has been shown to decrease mortality in adults with and without chronic medical conditions[32,33] and to have positive health benefits and improved quality of life for women with breast cancer.[34,35]

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CONFLICT OF INTEREST
NM is a cofounder of Sapere Bio, holds equity in the company, and is an inventor of intellectual property applications. The other authors have no conflicts to report.

DATA AVAILABILITY STATEMENT
Designated staff entered all study data into a REDCap™ database. All procedures were IRB and HIPAA-compliant. Database contains information for individual patients and is not publicly available. Investigators interested in these data should contact the corresponding author.

PATIENT CONSENT
Before recruitment and enrollment in this study, the patient was given a full explanation of the study and the opportunity to review the consent form. Informed, written consent was obtained from all participants in compliance with the Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

CLINICAL TRIAL REGISTRATION
The study was registered with clinicaltrials.gov (NCT02167932, NCT02328313, NCT03761706).

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