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Presence of crown-like structures in breast adipose tissue proximal and distant to tumor site among African American and White women with invasive breast cancer

By

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Master of Public Health

Global Epidemiology

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By

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B.S., University of Miami, 2019

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Abstract

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By Ellen L. Mitchell

Background: The etiologic drivers and clinical outcomes of crown-like structures in breast adipose tissue (CLS-B), a local marker of inflammation, within breast cancer patients remain largely under researched, especially among diverse populations. Inconsistencies in the methodologies used to detect and evaluate CLS-B likely contribute to the heterogenous findings.

Methods: CLS-B in tissue samples containing tumor (CLS-B_T) were assessed in 23 African American and 43 White women with invasive breast cancer. Tissue with tumor evidence was immunohistochemically stained for CD68 to visually assess CLS-B_T presence (yes vs. no). Patient demographics and clinical outcomes were abstracted from medical records and modeled in logistic regression as either risk factors (demographic and lifestyle information) or outcomes (tumor characteristics) of CLS-B_T to calculate odds ratios (ORs) and 95% confidence intervals (CIs). Associations between CLS-B_T presence and progression-free survival (PFS) were calculated as hazard ratios (HRs) with 95% CIs using Cox proportional hazards models. Results were compared to previous analyses of CLS-B detected in tissue samples uninvolved by tumor (CLS-B_{nT}).

Results: A weak association between race (African American vs. White) and CLS-B_T presence was observed in multivariable regression (OR = 0.54, 95% CI = 0.16, 1.82). Obesity (body mass index [BMI] ≥ 30) (OR = 1.49, 95% CI = 0.35, 6.41), age ≥ 60 at diagnosis (OR = 1.69, 95% CI = 0.47, 6.06) and having ever been a smoker (OR = 3.20, 95% CI = 1.01, 10.1) were positively associated with CLS-B_T presence. A weak, inverse association between CLS-B_T presence and PFS was observed (HR = 0.67, 95% CI = 0.12, 3.62). The percent agreement of CLS-B_T and CLS-B_{nT} within the sample of 66 was approximately 64%, and the percent agreement among those without CLS-B_{nT} or CLS-B_T was 74%.

Conclusions: CLS-B_T was not meaningfully different across demographic factors including race, but some evidence suggests that it is more common among women of older age, with increased adiposity, and among smokers. There is some evidence of a correlation between CLS-B_{nT} and CLS-B_T presence within the same study population. Standardized guidelines for CLS-B assessment are needed.

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Chapter I: Manuscript

Presence of crown-like structures in breast adipose tissue proximal and distant to tumor site among African American and White women with invasive breast cancer.

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Introduction

Obesity is an established risk factor for postmenopausal, hormone receptor (HR)-positive breast cancers, but inverse relationships between obesity and premenopausal, HR-positive breast cancers have also been observed.[1] Regardless of menopausal or HR status, obesity has been linked to poorer survival outcomes for breast cancer patients.[2,3] The underlying biological mechanisms between obesity and breast cancer risk and prognosis are not currently well understood. Metabolic and inflammatory changes within adipose tissue, an important endocrine organ, might play key roles in tumor development and progression.[4,5] While much of the previous epidemiological research has focused on the systemic effects of obesity on breast carcinogenesis, it is thought that tissue-based immune markers local to the tumor may provide more meaningful insight into the mechanisms of carcinogenesis.[6] Specifically, the breast adipose tissue microenvironment has recently become an important area of interest in investigation of the pathways between obesity and breast cancer.

Obesity-induced adipose inflammation and dysfunction occurs as adipose tissue, notably white adipose tissue (WAT), expands with weight gain.[3] Increased secretion of pro-inflammatory adipokines and cytokines by the adipose tissue, combined with decreased secretion of anti-inflammatory adipokines (adiponectin) characterizes this pro-inflammatory state.[5] These biomarkers of inflammation subsequently lead to insulin resistance, increased levels of circulating insulin and insulin-like growth factors (IGFs), and increased aromatization in adipose tissue.[5] Furthermore, adipose inflammation caused by expansion is associated with an infiltration of pro-inflammatory macrophages in the tissue microenvironment.[3,7] As adipocytes die, as a result of inflammation-induced hypoxia or by other means,[5] they become encircled by the

macrophages in a formation that resembles a crown.[3,8] These formations present pathologically as crown-like structures (CLS) and are a novel marker of WAT inflammation.[8] While breast adipose tissue is not as thoroughly understood as other subcutaneous or visceral fat depots, characteristic crown-like structures in breast adipose tissue (CLS-B) were first reported by Morris et al.[9] CLS-B continue to be studied as a histologic marker of local inflammation, but as reported in a recent review,[10] the clinical utility of CLS-B as it pertains to cancer risk, prognosis, and potential therapeutic interventions remains unclear.

Much of the inconsistency around CLS-B as a prognostic marker of breast cancer is related to differences in methods and approaches for CLS-B assessment. Standards for determining the location of where tissue specimens should be collected for analysis, in proximity to the tumor, to accurately and meaningfully ascertain CLS-B prevalence must be established from empirical evidence.[10] Most of the current research has utilized breast adipose tissue blocks that were distant to the cancer lesion, limiting the current capacity of CLS-B assessment to women who chose to undergo mastectomy. If CLS-B can be adequately measured from tumor specimen as opposed to tumor-free tissue specimen, the usefulness of CLS-B as a histologic marker for local inflammation will be greatly improved with increased application to a wider population.[10] There is a paucity of data on CLS-B within a tumor quadrant (denoted CLB-B_T). Specifically, the etiologic drivers CLS-B_T and associations with prognosis. There exist no studies which utilize and compare CLS-B_T and CLS-B within a non-tumor quadrant (CLS-B_{nT}) within the same patient population.

The present study aimed to address the unknowns around specimen selection for CLS-B assessment. We first estimated the association between patient (e.g., BMI, age, race, menopausal status) and CLS-B_T. Associations between CLS-B_T and tumor characteristics, including stage, grade, and tumor size were also investigated. Next, we estimated the association between CLS-B_T and cancer prognosis (overall survival [OS] and progression-free survival [PFS]). Importantly, for the first time, we calculated the correlation between CLS-B_T and CLS-B_{nT} within the same study population.

Methods

Study population

Cases were sampled as described by Maliniak et al.[11] Briefly, we identified cases via tumor registries associated with Emory University hospitals and included women aged ≥ 18 years that were diagnosed with primary invasive stage I-III breast cancer (ICD: C50) between January 1, 2007 and December 31, 2012. Following initial screening, cases were considered eligible if they were African American or White, underwent mastectomy at an affiliated hospital, and had never received neoadjuvant treatment for their breast cancer or systemic therapy for the treatment of a previous cancer diagnosis. Maliniak et al. included 342 patients that met the above criteria and that had available tissue specimens with adequate adipose tissue for CLS scoring and assessment.[11] Specimens could not have evidence of inflammation, tumor, or a biopsy tract. The present study represents a random subset of patients from this study population where we retrieved a representative tumor tissue block. Specimens from both a tumor quadrant and a non-tumor quadrant had to have evidence of adipose tissue that was adequate for CLS scoring and assessment. **Figure 1** illustrates patient selection and tissue availability for this study. In total, 66

participants (n = 23 African American, n = 43 white women) had the required specimens and were eligible to be included in final analysis. Participants provided informed consent at the time of their surgery at Emory University hospitals. This study was approved by the Institutional Review Board of Emory University (00100602).

Demographic and clinical data collection

Research staff at Emory University used REDCap (Research Electronic Data Capture) tools to abstract demographic and clinical characteristics for the study participants.[12,13] Demographic data included age a primary breast cancer diagnosis, height, weight, smoking status, family history of breast cancer among first-degree relatives, and concurrent medical conditions including diabetes, hypertension, and cholesterol status (high vs. low). Participant BMI was calculated using the provided height and weight data and was categorized using World Health Organization (WHO)-defined cut points: BMI < 25 kg/m^2 (under or ideal weight), BMI 25–29.9 kg/m^2 (overweight), or BMI ≥ 30 kg/m^2 (obese). Gynecologic and reproductive history were recorded as age at menarche, age at menopause, parity (nulliparous vs. parous), history of breastfeeding, menopausal status (pre-/perimenopausal vs. postmenopausal), and history of postmenopausal hormone replacement therapy use. A participant was classified as postmenopausal if they had record of a bilateral oophorectomy, reported permanent cessation of menses for 12 or more months (in the absence of chemotherapy or endocrine therapy), or were older than 55 years if data were missing. These data are reflective of the participant at their time of diagnosis, apart from height and weight which were recorded prior to mastectomy. Tumor characteristics and history of breast cancer treatments were abstracted from the time of surgery. Tumor stage (I, II, or III), tumor grade (well differentiated, moderately differentiated, or poorly

differentiated), estrogen receptor (ER) status (positive vs. negative), and the number of positive sentinel and axillary lymph nodes (0 vs. ≥ 1) were recorded. Tumor size, categorized as either < 1 centimeter or ≥ 1 centimeter, was also obtained. Tumor ER status was determined via staining by immunohistochemistry (IHC) (ER positive if > 1% staining by IHC). All demographic and clinical data were independently reviewed for quality assurance.

Biospecimen collection and evaluation

A standard tissue acquisition protocol was used to acquire all breast tissue specimens, including those containing tumor and those uninvaded by tumor. Between one and three formalin-fixed, paraffin-embedded (FFPE) blocks of breast tissue from a site distant to the cancer lesion and surrounding the lesion were obtained for each participant. All specimens were stained with hematoxylin and eosin (H&E) by a pathologist to differentiate the samples by tumor presence. The two best blocks per participant (one uninvolved with tumor and one with tumor presence) were selected based on the greatest area of WAT and minimal fat necrosis or increased inflammation.

Tissue blocks were prepared for CLS-B assessment using previously established methods as detailed by Maliniak et al.[11,14] Sections from the selected FFPE blocks were deparaffinized and rehydrated, and then stained for CD68 using IHC (Dako Envision (Dako) automated system for detection of CD68; 1:200 dilution, monoclonal mouse anti-human CD68 clone KP1, M0814, DAKO, Denmark) at Winship Cancer Institute's Pathology Core Laboratory (Emory University). Additional unstained tissue sections were cut between 100 and 200 μm of the original H&E-stained section from each respective block. Maliniak et al. describes the digital imaging and

CLS-B assessment process conducted on the non-tumor containing slides for the cohort of 342 participants.[11] For the present study, anti-CD68-immunostained slides were visually reviewed and scored by a pathologist (SGG) for the presence of CLS-B (yes vs. no) (**Figure 2**). CLS-B presence was defined as having ≥ 50% adipocyte encirclement. In both Maliniak et al. and the present study, pathologists and reviewers were masked to demographic characteristics, clinical data, and outcomes when scoring the tumor tissue specimens.[11] For the non-tumor containing specimens (n = 342), CLS-B density, measured as the number of CLS-B per square centimeter of breast WAT (CLS-B/cm^2) was assessed in addition to CLS-B presence.[11] The median CLS-B density of 0.87 CLS-B/cm^2 was used as the cut point between "low" and "high" CLS-B density.

Statistical analysis

Demographic and clinical characteristics of the study population, stratified by race and by CLS-B_T presence, were summarized by mean and standard deviation (SD) for continuous variables and by frequencies and percentages for categorical variables. Unadjusted and multivariable-adjusted logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for associations between various demographic and clinical factors and CLS-B_T presence. Demographic variables including race and BMI were treated as the exposure of interest for the dichotomous outcome of CLS-B_T presence. Alternatively, dichotomous clinicopathological variables, including ER status, tumor size, positive lymph nodes, and administered therapies, were treated as the outcome in regression models with CLS-B_T presence as the exposure. Ordinal outcomes of interest, specifically tumor stage and tumor grade, were analyzed using proportional odds models with the exposure variable of CLS-B_T presence. Potential confounders included in multivariable models were selected from previously

established associations in the literature and causal graphical analyses (directed acyclic graphs, DAGs). Identical regression models were additionally conducted using CLS-B_{nT} presence among the 66 participants for comparison. In addition, agreement statistics between CLS-B_{nT} and CLS-B_T presence (yes/no), and between CLS-B_{nT} density (high, low, or none) and CLS-B_T presence (yes/no) were calculated to assess the correlation between the two biomarkers within the study population (n = 66). Descriptive statistics were further utilized to investigate the demographic characteristics, namely race and BMI, among participants with both CLS-B_{nT} and CLS-B_T presence and participants with both CLS-B_{nT} and CLS-B_T absence.

To estimate the relationship of CLS-B and overall survival (OS), time from the date of diagnosis until death, last follow-up, or December 31, 2018, whichever came first, was calculated. For measurement of progression-free survival (PFS), time from the date of diagnosis to the date of breast cancer recurrence, determined via REDCap data abstraction, was additionally used. As described in Maliniak et al., mortality data was ascertained by the Georgia Center for Cancer Statistics via routine linkage with the state mortality file, the National Death Index, and through active follow-up/ other administrative sources.[11] Six participants were excluded from survival analyses: three were not Georgia residents and three had missing covariate data. Analyses of survival outcomes by CLS-B_T presence were conducted using age- and multivariable-adjusted Cox proportional hazards regression models and examined using Kaplan-Meier curves and the log-rank test. Hazards ratios (HRs) and 95% confidence intervals (CIs) are reported from the regression models. Existing literature and DAG analyses showed that age of diagnosis, BMI, and smoking status were associated with both CLS-B presence and clinical outcomes, and therefore were included in multivariable models.

Results

Study population

The distribution of demographic and clinical characteristics at time of diagnosis for the 66 participants compared to the data for the full CLS-B_{nT} cohort (n = 342) is provided in **Table 1**. The present study population comprises 23 (34.8%) African American women and 43 (65.2%) White women. The mean age at diagnosis among African American women was 57.9 years (SD 12.52) and was higher than the mean age of 53.5 (SD 11.76) among White women. Among African American participants, 5 (21.7%) had a BMI less than 25, 7 (30.4%) had a BMI between 25 and 30, and 11 (47.8%) had a BMI greater than or equal to 30. This contrasts with the distribution of BMI among White participants, where 26 (60.5%) had a BMI less than 25, 8 (18.6%) between 25 and 30, and 9 (17.3%) greater than or equal to 30. More than half of the participants were postmenopausal (61.9% of African American participants and 55.1% of White participants) at the time of diagnosis. The distribution of ER-positive cancer was higher among White women than African American women (83.7% vs. 69.6%) compared with ER-negative tumors. This likely contributes to the finding that White women were also more likely to receive hormone therapy (81.4% vs. 65.2%). The proportion of participants diagnosed with stage I cancer was higher among White women than African American women (53.5% vs. 39.1%), while the proportion of stage II and stage III diagnoses were higher among African American women (47.8% vs. 41.9% and 13% vs. 4.7%). African American participants were also more likely to have poorly differentiated tumors than White participants (39.1% vs. 16.3%). Of the 20 participants that were found to have CLS-B_T presence, 14 (70%) were White and 6 (30%) were African American. Similarly, White participants made up 63% of those without any CLS-B_T, while 37% were African American. Among participants with CLS-B_T presence, 60% had a BMI

of 25 or higher (overweight and obese categories). **Figure 3** depicts the relationship between BMI, CLS-B_T presence, and race.

CLS-B_T presence by patient characteristics at diagnosis

Multivariable models adjusting for race and BMI show that participants aged 60 or older at the time of diagnosis were more likely to have CLS-B_T presence than participants between the ages of 50 and 60 when compared with participants less than 50 years of age (OR = 1.69, 95% CI = 0.47, 6.06 vs. OR = 1.02, 95% CI = 0.24, 4.26) (**Table 2**). Before adjusting for potential confounders, participants with a BMI of 30 or greater (classified as obese) were almost twice as likely to have any CLS-B_T presence compared with participants with a BMI less than 30 (classified as either overweight, ideal weight, or underweight) (OR = 1.92, 95% CI = 0.58, 6.38). Adjustment for age at diagnosis, smoking status, and race attenuated this association, but the findings are still noteworthy (OR = 1.49, 95% CI = 0.35, 6.41).

Past smokers were 3 times more likely to have CLS-B_T than never smokers in the study population (OR = 3.20, 95% CI = 1.01, 10.14). There is no evidence of a relationship between age at menarche and CLS-B_T in this study population (OR = 0.95, 95% CI = 0.65, 1.40). An inverse relationship between history of breastfeeding and CLS-B_T was observed (OR = 0.46, 95% CI = 0.10, 2.04). Additionally, postmenopausal participants were more likely to have CLS-B_T presence when compared to pre- and perimenopausal participants, following adjustment for age at diagnosis, race, and BMI (OR = 1.76, 95% CI = 0.31, 10.0). Associations between CLS-B_{nT} and patient characteristics among the 66 participants with CLS-B_T data are provided in **Supplementary Table 1.**

Tumor characteristics and clinical outcomes by CLS-B_T presence

Table 3 provides frequencies and estimates of tumor characteristics that were treated as outcomes in analysis of CLS-B_T exposure. Following adjustment for age at diagnosis, BMI, and race, we did not observe an association between higher tumor stage (stage II vs. stage I) and CLS-B_T presence (OR = 1.17, 95% CI = 0.37, 3.70). Frequencies and estimates of tumor characteristics by CLS-B_{nT} exposure are provided in **Supplementary Table 2.**

The 60 participants included for survival analyses were followed for an average of 7.1 years (SD = 1.9), during which 2 participants died and 7 participants had evidence of breast cancer recurrence. CLS-B_T presence was found to have an inverse association with PFS following adjustment for age at diagnosis, BMI, and smoking status (HR = 0.67, 95% CI = 0.12, 3.62) but the association was imprecise and included the null (**Figure 4**).

Agreement between CLS-B_T and CLS-B_{nT}

The overall percent agreement between CLS-B_{nT} and CLS-B_T within the study population was 63.6% (42/66, **Table 4).** Among those without CLS-B_T presence, 73.9% (34/46) also did not have evidence of CLS-B_{nT}. Of the participants previously established to have CLS-B_{nT} presence, 40% (8/20) also showed evidence of CLS-B_T. Among the eight participants with CLS-B in both the tumor and non-tumor tissues, 6 (75%) were classified as having high CLS-B_{nT} density in previous analysis. Furthermore, more than half (62.5%) of the participants with both CLS-B_{nT} and CLS-B_T were categorized as obese (BMI $\geq$ 30). Comparingly, 50% of the 34 participants exhibiting no CLS-B presence at either site had BMI < 25.

Discussion

Despite having a limited study population, the present study contributes new knowledge about CLS-B_T presence and ascertainment to the growing body of CLS-B research. Among the 66 participants included in analysis, 20 (30.3%) had evidence of CLS-B_T. Interestingly, an identical proportion of the study population were found to have CLS-B_{nT}. Nonetheless, just 8 participants (12.1%) had evidence of CLS-B at both tissue locations and 34 (51.5%) did not have CLS-B at either location. Overall, we found a weak inverse relationship between African American race (compared with White race) and CLS-B_T in this population, but it is likely that this finding is driven by small numbers. Most evidence from previous studies support that CLS-B is not meaningfully different across racial/ethnic groups.[11,15,16] Yet, one study found that Black women, compared with non-Black Latina and Caucasian, presented with the highest frequencies of CLS-B_T.[17] However, this study stained tumor tissue for macrophage markers other than CD68 and did not control for BMI in analysis.[17] As results of the present study were imprecise, larger studies with better statistical power are required to draw definitive conclusions.

In alignment with previous epidemiologic research of CLS-B assessed in differing breast adipose tissue sites,[11,14-23] we observed an increased association of CLS-B_T with increased BMI. Specifically, participants in the highest BMI category (BMI ≥ 30) were more likely to have CLS-B_T presence than those with BMI scores under 25. Most of the participants exhibiting CLS-B in sites both distant and proximal to the tumor were in the obese category (62.5%), providing evidence that the association with BMI is seen throughout the breast adipose tissue environment. This observation is consistent with previous evidence from a Norwegian cross-sectional study that found a positive relationship between BMI and CLS-B in adipose tissue close to the tumor.[19]

Both investigations, however, were among small study populations, and further research of CLS-B_T among large, diverse cohorts is warranted. Despite this association, CLS-B_T was still observed in participants of under or ideal weight (BMI < 25 kg/m^2) in 6/20 participants with CLS-B_T presence, indicating that adiposity might not be the only driver of CLS-B. This finding is consistent with results from a previous study of CLS-B_{nT} detection among 72 U.S. women with normal BMIs (BMI < 25 kg/m^2) who underwent mastectomy for breast cancer risk reduction or treatment.[23]

For the first time, this study provides data on the relationship between CLS-B_T and CLS-B_{nT} within a single, diverse cohort. Agreement statistics calculated between CLS-B_T presence and CLS-B_{nT} presence and densities provide evidence that the biomarker in one location could be indicative of the biomarker in another location with the breast adipose tissue (% agreement of CLS presence = 63.6%). It was also observed that 73.9% of participants without CLS-B_T also did not have any CLS-B_{nT} presence. An absence of CLS-B in a tumor-invaded quadrant may be indicative of an absence of CLS-B in other areas of breast adipose tissue for a patient, but further investigation is required. Assessment of CLS-B_T density, as opposed to a simple yes vs. no presence assessment, would benefit future studies of this relationship. Due to biospecimen collection restraints, assessment of CLS-B within tumor samples has greater potential clinical utility compared with the assessment of breast tissue distant to the tumor. If CLS-B_T is representative of CLS-B elsewhere in the breast tissue, analysis could be conducted on larger samples of women undergoing breast conserving surgeries (*e.g.*, lumpectomy) as opposed to the current constraint to mastectomy samples.

The observed differences in CLS-B presence between tissue quadrants proximal and distal to the lesion may also suggest that more than one tissue sample per patient is required for CLS-B assessment. Currently, no guidelines on the adequate tissue sampling for CLS-B assessment exist.[10] Previous studies have utilized differing numbers of selected tissue blocks, and number of samples per block, for each participant, with one study assessing a median 7 blocks per patient.[20] This study, however, differentially selected specimen with the highest number of CLS-B to be used in analysis, which would contribute to stronger effect estimates. The clinical utility of CLS-B assessment diminishes as more samples per participant are required, but evidence from the present study suggests that one sample is likely not sufficient. Larger studies with multiple samples from breast tissue proximal to the cancer lesion are required.

Despite CLS-B_{nT} and CLS-B_T being previously associated with worse clinical outcomes among breast cancer patients,[17,20,24] the present study observed a weak inverse relationship between CLS-B_T and PFS. However, results were imprecise and subject to numerous biases. Among the full cohort of 342 breast cancer patients, CLS-B_{nT} was not found to have any relationship with PFS or OS.[11] One recent study found robust associations between CLS-B_{nT} and poorer clinical outcomes (distant recurrence and overall mortality) independent of BMI and tumor factors, but notably, researchers differentially selected tissue samples with the highest frequencies of CLS-B_{nT}.[20] A second study that observed similar associations used different macrophage markers (CD163, CD206, and CD40) for CLS-B assessment than customarily used.[17] The differences observed in clinical outcomes likely results from the heterogeneity of CLS-B assessment methodologies.

The present study has several important considerations. The sample size was small and statistical power was limited, yielding imprecise estimates. Additionally, samples of African American and White participants were not equal, limiting our ability to detect differences in the two groups The use of only one anti-CD68-stained adipose tissue slide per participant and the evaluation of CLS-B_T presence only (yes vs. no) likely created non-differential misclassification of the exposure when assessing the impact of CLS on tumor characteristics and clinical outcomes, and non-differential misclassification of the outcome when examining potential etiologic drivers of CLS-B_T. We expect this probable misclassification to bias results towards the null, potentially masking important associations. Despite these limitations, the present study was able to produce agreement statistics between CLS-B_{nT} and CLS-B_T using identical methodologies within the same study population. This novel research can guide future investigations CLS-B assessment processes and strategies and may contribute to the standardization of CLS-B detection guidelines.

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Table 1. Demographic and clinical characteristics of 342 women diagnosed with invasive breast cancer (stages I-III) and a subset of 66 women by race, Emory University, 2007-2012

			Race	
	CLS-B_{nT} Cohort (N = 342)	CLS-B_T Sample (N = 66)	CLS-B_T African American (N = 23)	CLS-B_T White (N = 43)
Demographic characteristics				
Age at diagnosis (years)				
Mean (SD)	55.3 (13.2)	55.0 (12.1)	57.9 (12.5)	53.5 (11.8)
BMI, *n*(%)				
< 25	126 (37.1)	31 (47.0)	5 (21.7)	26 (60.5)
25 to < 30	85 (25.0)	15 (22.7)	7 (30.4)	8 (18.6)
≥ 30	129 (37.9)	20 (30.3)	11 (47.8)	9 (20.9)
Smoking status, *n*(%)				
Non-smoker	225 (67.0)	40 (63.5)	15 (68.2)	25 (61.0)
Past smoker	87 (25.9)	21 (33.3)	6 (27.3)	15 (36.6)
Current smoker	24 (7.1)	2 (3.2)	1 (4.6)	1 (2.4)
Age at menarche (years)				
Mean (SD)	12.7 (1.7)	12.9 (1.5)	12.8 (1.4)	13.0 (1.6)
Parity, *n*(%)				
Nulliparous	44 (15.0)	11 (17.5)	3 (14.3)	8 (19.1)
Parous (1+ live births)	249 (85.0)	52 (82.5)	18 (85.7)	34 (81.0)
History of breastfeeding[a], *n*(%)				
No	75 (39.5)	15 (32.6)	6 (42.9)	9 (28.1)
Yes	115 (60.5)	31 (67.4)	8 (57.1)	23 (71.9)
Menopausal status, *n*(%)				
Pre-/perimenopausal	117 (36.1)	27 (42.2)	8 (38.1)	19 (44.2)
Postmenopausal	207 (63.9)	37 (57.8)	13 (61.9)	24 (55.8)
Age at menopause[b], *n*(%)				
< 45	83 (45.4)	18 (50.0)	9 (75.0)	9 (37.5)
45 to <50	35 (19.1)	8 (22.2)	2 (16.7)	6 (25.0)
50 to < 55	50 (27.3)	7 (19.4)	1 (8.3)	6 (25.0)
≥ 55	15 (8.2)	3 (8.3)	0 (0.0)	3 (12.5)
History of postmenopausal hormone replacement therapy use[b], *n*(%)				
No	120 (63.8)	19 (55.9)	7 (63.6)	12 (52.2)
Yes	68 (36.2)	15 (44.1)	4 (36.4)	11 (47.8)
Family history of breast cancer among first-degree relatives, *n*(%)				
No	240 (73.6)	47 (75.8)	13 (68.4)	34 (79.1)
Yes	86 (26.4)	15 (24.2)	6 (31.6)	9 (20.9)
Diabetes status, *n*(%)				
No	298 (87.1)	59 (89.4)	18 (78.3)	41 (95.4)
Yes	44 (12.9)	7 (10.6)	5 (21.7)	2 (4.7)
Hypertension status, *n*(%)				
No	200 (58.5)	41 (62.1)	10 (43.5)	31 (72.1)
Yes	142 (41.5)	25 (37.9)	13 (56.5)	12 (27.9)
High cholesterol status, *n*(%)				
No	286 (83.6)	58 (87.9)	19 (82.6)	39 (90.7)
Yes	56 (16.4)	8 (12.1)	4 (17.4)	4 (9.3)
Clinical characteristics				
ER status, *n*(%)				
Positive	267 (79.0)	52 (78.8)	16 (69.6)	36 (83.7)
Negative	71 (21.0)	14 (21.2)	7 (30.4)	7 (16.3)
Stage, *n*(%)				
Stage I	163 (47.7)	32 (48.5)	9 (39.1)	23 (53.5)
Stage II	135 (39.5)	29 (43.9)	11 (47.8)	18 (41.9)
Stage III	44 (12.9)	5 (7.6)	3 (13.0)	2 (4.7)
Tumor grade, *n*(%)				

Well differentiated	75 (22.9)	12 (18.2)	4 (17.4)	8 (18.6)
Moderately differentiated	150 (45.7)	38 (57.6)	10 (43.5)	28 (65.1)
Poorly differentiated	103 (31.4)	16 (24.2)	9 (39.1)	7 (16.3)
Tumor size (cm), *n*(%)				
< 1	50 (16.8)	10 (16.7)	1 (5.3)	9 (22.0)
≥ 1	247 (83.2)	50 (83.3)	18 (94.7)	32 (78.1)
Number of positive lymph nodes, *n*(%)				
0	204 (65.6)	44 (68.8)	12 (57.1)	32 (74.4)
≥ 1	107 (34.4)	20 (31.3)	9 (42.9)	11 (25.6)
Chemotherapy, *n*(%)				
No	172 (52.4)	35 (53.9)	9 (40.9)	26 (60.5)
Yes	156 (47.6)	30 (46.2)	13 (59.1)	17 (39.5)
Radiation, *n*(%)				
No	235 (71.4)	48 (75.0)	13 (61.9)	35 (81.4)
Yes	94 (28.6)	16 (25.0)	8 (38.1)	8 (18.6)
Hormone Therapy, *n*(%)				
No	109 (31.9)	16 (24.2)	8 (34.8)	8 (18.6)
Yes	233 (68.1)	50 (75.8)	15 (65.2)	35 (81.4)

Abbreviations: *BMI* body mass index, *SD* standard deviation

Note: Missing values were not included in the calculation of percentages

[a]Among parous women

[b]Among postmenopausal women

Table 2. Associations of potential risk factors with detection of crown-like structures in breast adipose tissue surrounding a tumor (CLS-B_T) among 66 women diagnosed with invasive breast cancer (stages I-III), Emory University, 2007-2012

	Any CLS-B_T[a]			
	No N = 46	Yes N = 20	Unadjusted OR[b] (95% CI)	Adjusted OR[b] (95% CI)
Characteristic	**N (%)**	**N (%)**		
Race[c]				
White	29 (63.0)	14 (70.0)	1.00 (-)	1.00 (-)
African American	17 (37.0)	6 (30.0)	0.73 (0.24-2.26)	0.54 (0.16-1.82)
Age at diagnosis (years)[c]				
25 to < 50	17 (37.0)	6 (30.0)	1.00 (-)	1.00 (-)
50 to < 60	13 (28.2)	5 (25.0)	1.09 (0.27-4.37)	1.02 (0.24-4.26)
≥ 60	16 (34.8)	9 (45.0)	1.59 (0.46-5.50)	1.69 (0.47-6.06)
BMI (kg/m^2)[c,g]				
< 25	23 (50.0)	8 (40.0)	1.00 (-)	1.00 (-)
25 to < 30	11 (23.9)	4 (20.0)	-	-
≥ 30	12 (26.1)	8 (40.0)	1.92 (0.58-6.38)	1.49 (0.35-6.41)
Smoking status[c,g]				
Non-smoker	31 (70.5)	9 (47.4)	1.00 (-)	1.00 (-)
Past smoker	11 (25.0)	10 (52.7)	3.13 (1.01-9.72)	3.20 (1.01-10.14)
Current smoker	2 (4.6)	0 (0)	-	-
Age at menarche (years)[c,d]	13.0 (1.7)	12.8 (1.1)	0.89 (0.62-1.3)	0.95 (0.65-1.40)
Parity[c,g]				
Nulliparous	8 (18.6)	3 (15.0)	-	-
Parous (1+ live births)	35 (81.4)	17 (85.0)	-	-
History of breastfeeding[c,e]				
No	9 (28.1)	6 (42.9)	1.00 (-)	1.00 (-)
Yes	23 (71.9)	8 (57.1)	0.52 (0.14-1.93)	0.46 (0.10-2.04)
Menopausal status[c]				
Pre-/perimenopausal	19 (43.2)	8 (40.0)	1.00 (-)	1.00 (-)
Postmenopausal	25 (56.8)	12 (60.0)	0.88 (0.30-2.57)	1.76 (0.31-10.04)
Age at menopause[c,f,g]				
< 45	11 (45.8)	7 (58.3)	-	-
45 to <50	4 (16.7)	4 (33.3)	-	-
50 to < 55	7 (29.2)	0 (0)	-	-
≥ 55	2 (8.3)	1 (8.3)	-	-
Hormone replacement therapy use[c,f]				
No	13 (56.5)	6 (54.6)	1.00 (-)	1.00 (-)
Yes	10 (43.5)	5 (45.5)	1.08 (0.26-4.60)	1.39 (0.25-7.77)
Family history of breast cancer[c]				
No	32 (76.2)	15 (75.0)	1.00 (-)	1.00 (-)
Yes	10 (23.8)	5 (25.0)	1.07 (0.31-3.67)	1.37 (0.37-5.02)
Diabetes mellitus[c,g]				
No	41 (89.1)	18 (90.0)	-	-
Yes	5 (10.9)	2 (10.0)	-	-
Hypertension[c]				
No	30 (65.2)	11 (55.0)	1.00 (-)	1.00 (-)
Yes	16 (34.8)	9 (45.0)	1.53 (0.53-4.47)	1.40 (0.42-4.65)
High cholesterol[g]				
No	43 (93.5)	15 (75.0)	-	-
Yes	3 (6.5)	5 (25.0)	-	-

Abbreviations: *BMI* body mass index, *CI* confidence interval, *CLS-B_T* crown-like structures in the breast near tumor, *OR* odds ratio

Note: Missing values were not included in the calculation of percentages

[a]Any CLS-B_T was defined as detection of ≥ 1 CLS-B_T with adipocyte encirclement ≥ 50% on the tissue section examined

[b]Estimated odds ratios using logistic regression models with CLS-B_T presence (yes versus no) as the outcome

[c]All models were adjusted for age at diagnosis (years), race (African American, white), and body mass index (kg/m^2) except for those models where these covariates were the exposures of interest in which case covariates were mutually adjusted for; BMI models additionally adjusted for smoking status (never smoker, past smoker, current smoker)
[d]Values expressed as mean (standard deviation)
[e]Among parous women
[f]Among postmenopausal women
[g]No estimated ORs (95% CIs) where data counts < 5 participants

Table 3. Associations between detection of crown-like structures in breast adipose tissue surrounding a tumor (CLS-B_T) and tumor characteristics among 66 women diagnosed with invasive breast cancer (stages I-III), Emory University, 2007-2012

	Any CLS-B_T[a]	
	No (N = 46)	**Yes (N = 20)**
Clinical characteristic		
ER status		
Positive	35 (76.1)	17 (85.0)
Negative	11 (23.9)	3 (15.0)
Stage		
Stage I	23 (50.0)	9 (45.0)
Stage II	19 (41.3)	10 (50.0)
Stage III	4 (8.7)	1 (5.0)
Tumor grade		
Well differentiated	9 (19.6)	3 (15.0)
Moderately differentiated	23 (50.0)	15 (75.0)
Poorly differentiated	14 (30.4)	2 (10.0)
Tumor size (cm)		
< 1 cm	7 (17.1)	3 (15.8)
≥ 1 cm	34 (82.9)	16 (84.2)
Number of positive lymph nodes		
0	29 (64.4)	15 (79.0)
≥ 1	16 (35.6)	4 (21.1)
	Any CLS-B_T vs. None	
	Unadjusted OR (95% CI)	**Adjusted OR (95% CI)**
Outcome		
Stage II cancer (vs. Stage I)	1.35 (0.45-3.99)	1.17 (0.37-3.70)

Abbreviations: *CI* confidence interval, *CLS-B_T* crown-like structures in the breast near tumor, *OR* odds ratio

Note: missing values not included in the calculation of percentages

[a]Any CLS-B_T was defined as detection of ≥ 1 CLS-B_T with adipocyte encirclement ≥ 50% on the tissue section examined

Table 4. Correlation between crown-like structures in breast adipose tissue surrounding a tumor (CLS-B_T) and crown-like structures in breast adipose tissue uninvaded by tumor (CLS-B_{nT}) among 66 women diagnosed with invasive breast cancer (stages I-III), Emory University, 2007-2012

		CLS-B_{nT} Presence[b]		
		Yes	No	Total
CLS-B_T Presence[a]	Yes	8	12	20
	No	12	34	46
	Total	20	46	66

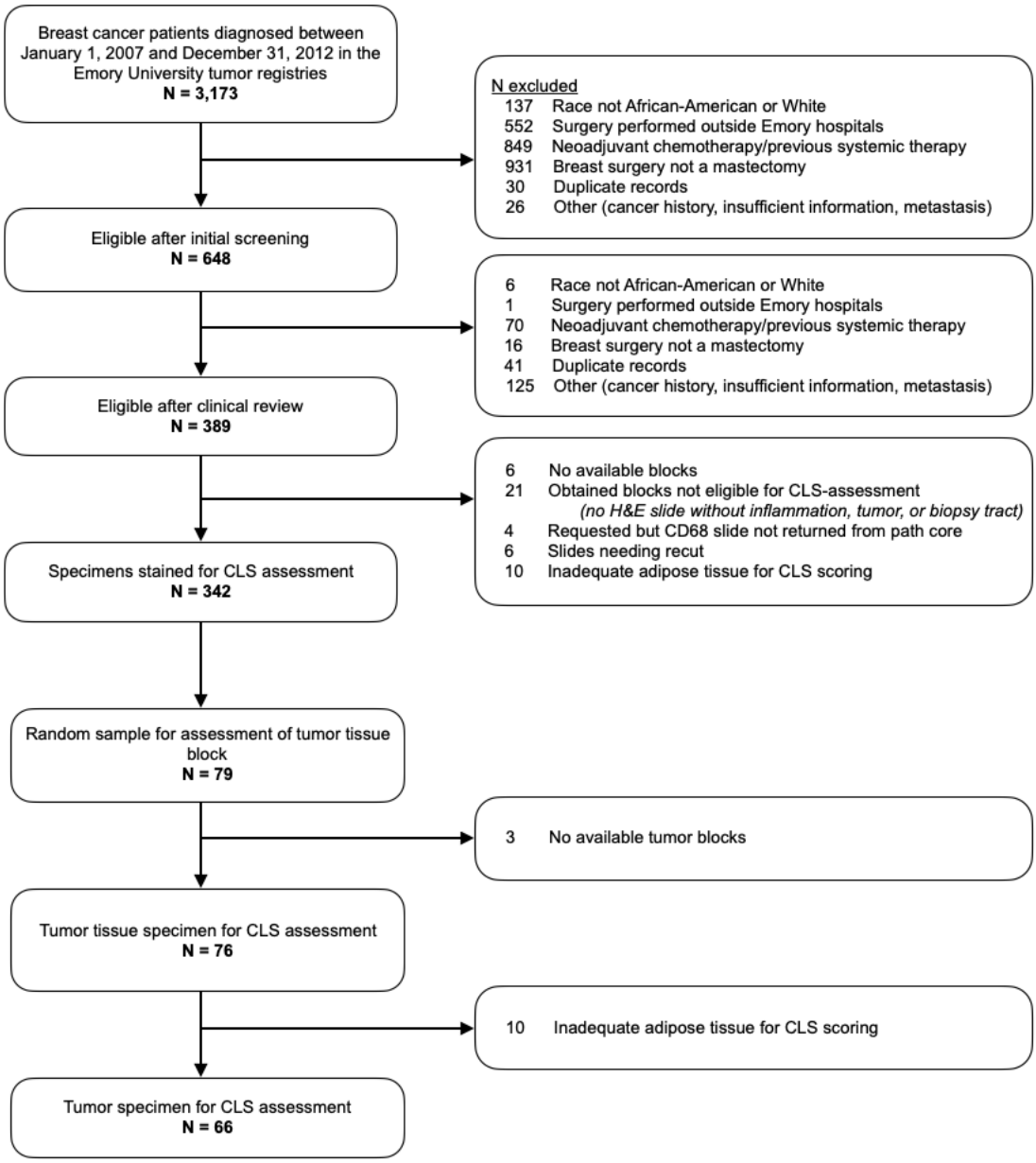
Abbreviations: *CLS-B_T* crown-like structures in the breast near tumor, *CLS-B_{nT}* crown-like structures in the breast distant to tumor
[a]Any CLS-B_T was defined as detection of ≥ 1 CLS-B_T with adipocyte encirclement ≥ 50% on the tissue section examined
[b]Any CLS-B_{nT} was defined as detection of ≥ 1 CLS-B_{nT} with adipocyte encirclement ≥ 50% on the tissue section examined

Table 5. Correlation between crown-like structures in breast adipose tissue surrounding a tumor (CLS-B_T) and the density of crown-like structures in breast adipose tissue uninvaded by tumor (CLS-B_{nT}) among 66 women diagnosed with invasive breast cancer (stages I-III), Emory University, 2007-2012

		CLS-B_{nT} Density[b]			
		High[c]	Low[d]	None	Total
CLS-B_T Presence[a]	Yes	6	2	12	20
	No	7	5	34	46
	Total	13	7	46	66

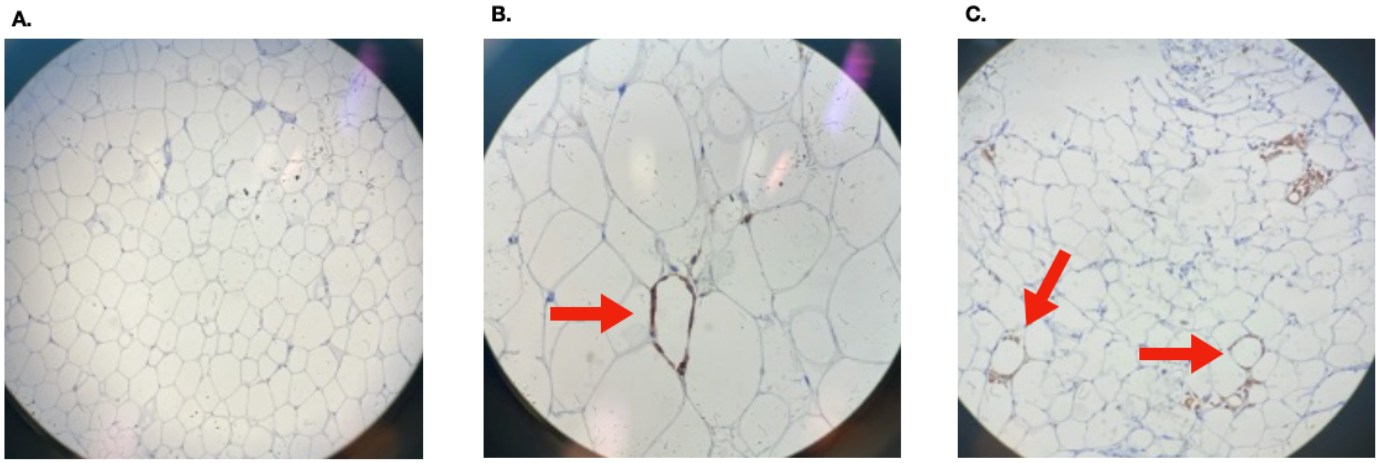
Abbreviations: *CLS-B_T* crown-like structures in the breast near tumor, *CLS-B_{nT}* crown-like structures in the breast distant to tumor
[a]Any CLS-B_T was defined as detection of ≥ 1 CLS-B_T with adipocyte encirclement ≥ 50% on the tissue section examined
[b]CLS-B_{nT} density measured in CLS-B_{nT} with adipocyte encirclement ≥ 50% on the tissue section examined per cm^2 of adipose tissue
[c]High density defined as ≥ 0.87 CLS-B_{nT}/cm^2
[d]Low density defined as < 0.87 CLS-B_{nT}/cm^2

Figure 1. Flow chart illustrating patient selection and tissue availability



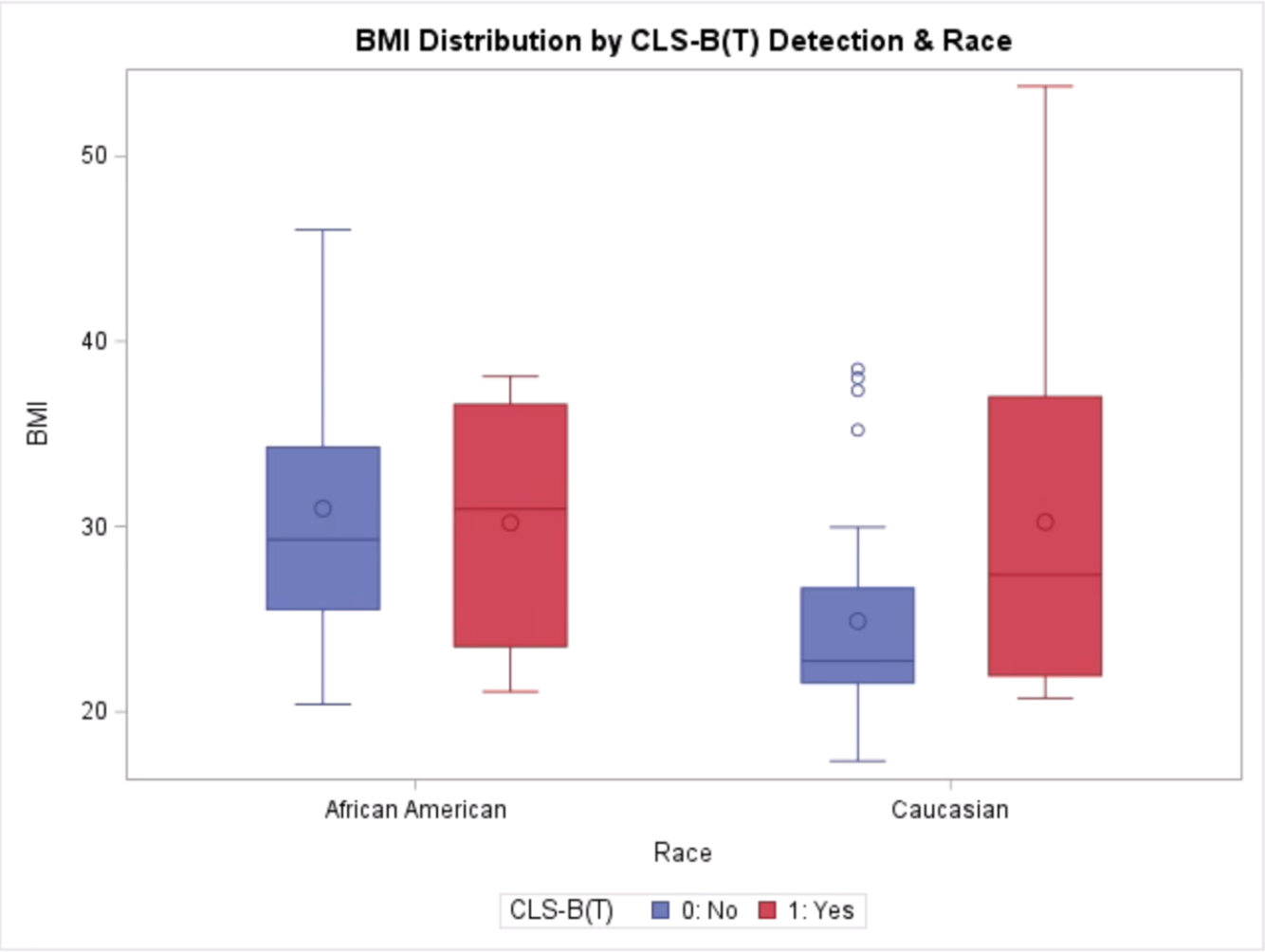
Note: Specimen stained for CLS-assessment (n = 342) derived from Maliniak et al.[11]

Figure 2. Example images of anti-CD68-immunostained slides of breast WAT surrounding the tumor within the study sample



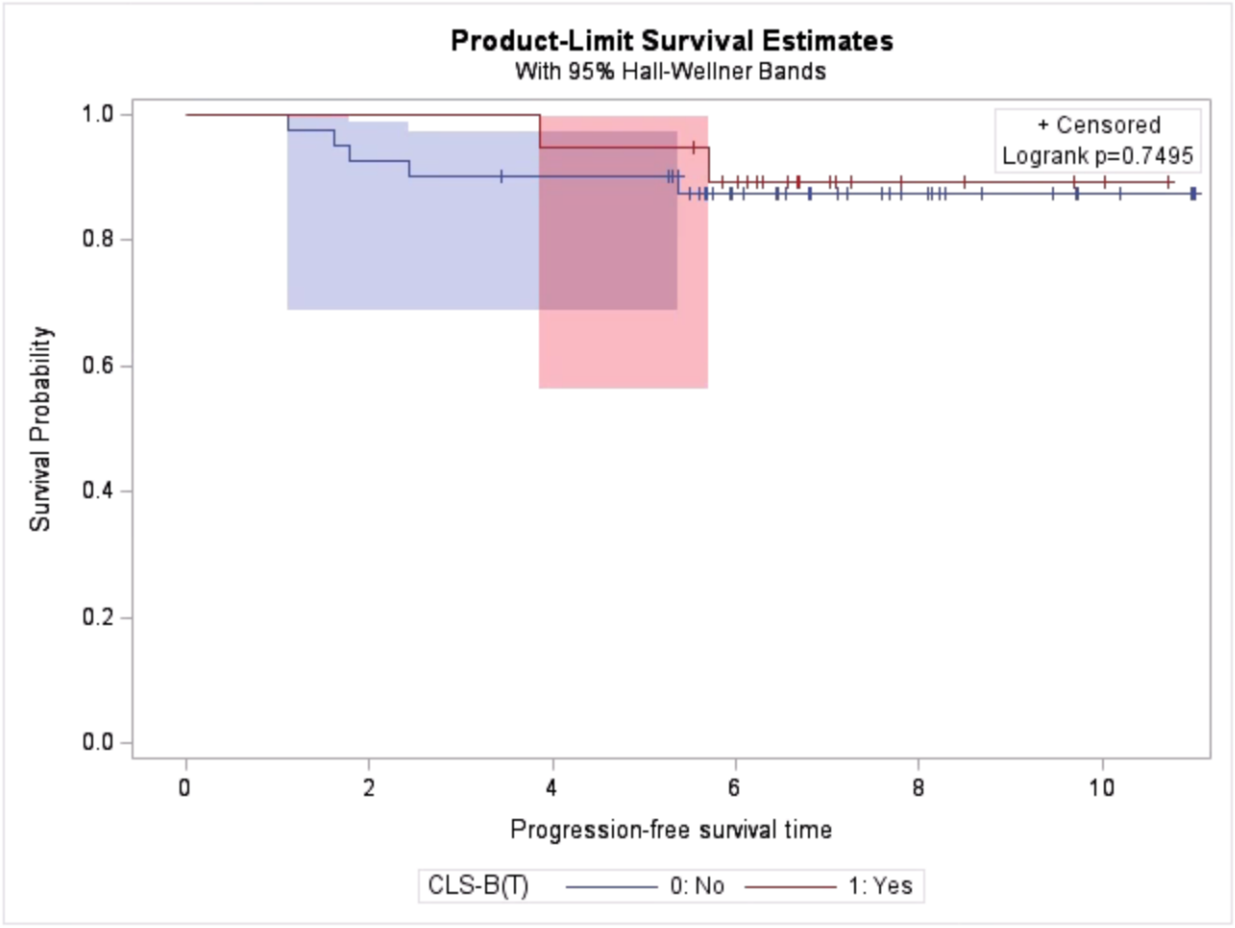
a Breast WAT without evidence of CLS-B_T. **b** Breast WAT with evidence of one CLS-B_T (≥ 90% adipocyte encirclement). **c** Breast WAT with evidence of multiple CLS-B_T.

Figure 3.



Abbreviations: *BMI* body mass index (kg/m^2), *CLS-B(T)* crown-like structures in the breast near tumor

Figure 4. Kaplan-Meier curves comparing progression-free survival by detection of crown-like structures in breast adipose tissue surrounding a tumor (CLS-B_T) among 66 women diagnosed with invasive breast cancer (stages I-III), Emory University, 2007-2012



Abbreviations: *CLS-B(T)* crown-like structures in the breast near tumor

Chapter II: Extended Results

Literature Review

Author, Year PMID	Population		Research Question	CLS Assessment		Statistical analysis	Results (report main effect estimates only)	Overarching conclusions
	N Cancer Stage	geography race/ethnicity		Staining/ Antibody	Quadrant(s) analyzed	(General notes)		
Brown, 2017 28323914	102 premenopausal women 59 postmenopausal women total = 161 All underwent mastectomy for the prevention or treatment of breast cancer (invasive tumor present n=128, no invasive tumor present n=29, missing n=4)	United States, Memorial Sloan Kettering Cancer Center (MSKCC) Asian (n=7), Black (n=13), Other (n=4), White (n=122), Missing (n=15)	Does menopause affect breast aromatase expression? What is the effect of menopause on breast aromatase expression in relation to BMI, white adipose tissue inflammation (WATi), and systemic markers of metabolic dysfunction (CLS)	immunohistochemistry for CD68	Breast tissue uninvolved by tumor, 5 sections per case	Clinicopathologic feature between pre- and postmenopausal patients examined using nonparametric Wilcoxon rank-sum test for continuous variables and Fisher's exact test or Chi-squared for categorical features Multiple linear regression was used to assess the association between continuous variables and menopausal status, adjusting for other covariates (BMI and/or CLS-B)	Menopause associated with a higher proportion of CLS-B+ cases (42 of the 59 cases, or 71%) compared with premenopausal women (51 of the 102 cases, or 50%; P = 0.01). Aromatase was correlated with CLS-B/cm^2 in both pre- and postmenopausal women, but the association was significantly stronger in postmenopausal women (P = 0.001).	Various obesity-related parameters are more strongly associated with aromatase after menopause. Higher aromatase levels in inflamed breast adipose tissue is a plausible mechanism for the higher incidence of hormone-dependent breast cancer in obese women after menopause.
Carter, 2017 29167285	258 3, age-matched groups of 86 each 86 normal breast tissue donors from KTB, 86 with initial benign biopsy to subsequently developed cancer (BBD cases), 86 BBD subjects with benign biopsies who did not develop breast cancer (BBD controls)	Mayo Benign Breast Disease Cohort and donor breast tissues from the Komen Tissue Bank (KTB) NR	1. The association of CLS-B microenvironment in normal donor and BBD tissues with clinical parameters including BMI and age 2. The association of CLS-B microenvironment in normal donor and BBD tissues with established pathologic risk factors of breast cancer (including histologic categories of BBD and lobular involution)	Stained with hematoxylin and eosin (H&E) and for the macrophage marker, CD68	One block/sample stained for CD68, unstained tissue sections sequentially cut from the corresponding tissue blocks within an estimated 100-200 micrometers of original H&E stained section	Conditional logistic regression used to examine associated with BBD cases vs controls by univariate and multivariate analysis. Negative binomial regression used for comparisons with KTB group	BBD cases more likely to have CLS-B than BBD controls (24.4% vs. 18.6% with any CLS-B, *P* = 0.07). High numbers of CLS-B were an independent risk factor for breast cancer after adjusting for adipose area, histologic impression, and BMI (OR=6.8, *P* = 0.02). CLS-B strongly associated with BMI.	BBD stromal tissues are more frequently inflamed than donor breast tissues. CLS-B-associated adipose tissue inflammation occurs in a significant subset of individuals with BBD. Findings also suggest that it is the extent or quantity of CLS-B inflammation in BBD tissues, rather than its presence or absence, that may predict breast cancer risk.

Cha, 2018 29468486	56 from reduction mammoplasty (Group 1) 84 non-neoplastic breast tissue of breast cancer patients (Group 2) 140 breast cancer with adipose stroma (Group 3) Groups 2 and 3 had tumor stages ranging from I to III	NR NR	To evaluate macrophage infiltration and assess the presence of CLS in mammary adipose tissue in cases with and without breast cancer	Stained with hematoxylin and eosin (H&E) and for macrophage markers CD68 and CD163	Available tissue samples - For Group 2, resection margin tissue was used. For Group 3, breast cancer tissue harboring predominant adipose stroma (greater than 50% of stroma is adipose tissue) was used	*t*-tests and chi-squared test used for continuous and categorical variables, respectively. Kaplan-Meier survival curves and log-rank tests used to evaluate time to tumor recurrence, DFS, and OS. Multivariate regression analysis using Cox proportional hazards model were utilized	Group 3 had the most positive macrophages and CLS in adipose tissue (p<0.001). Among Group 3, levels of macrophages were related to higher histologic grade and CLS were correlated with old age (p = 0.042). CLS were associated with shorter OS in node-positive breast cancer patients (p=0.015)	CD68+ and/or CD163+ tumor-associated macrophage infiltration as well as CLSs are present in adipose tissue nearby the breast cancer lesion, and are associated with various clinicopathologic parameters of breast cancer
Chang, 2021 34294716	H&E slides: n=163 CD68 stained slides: n=119 Some had partial mastectomy, some had total mastectomy surgically resected early breast cancer (T1 to T3, N0 or N1, M0)	Mount Sinai Hospital, Toronto NR	To examine breast adipose tissue for CLS-B, and compare CD68 immunohistochemistry to standard histology (H&E staining), and examine associations between CLS-B and patient and tumor characteristics, metabolic factors, and survival	Stained with hematoxylin and eosin (H&E) and for the macrophage marker, CD68 hCLS-B = CLS assessed with H&E staining CD68+ CLS-B = CLS assessed with CD68 macrophage marker	Quadrants without tumor, median 7 blocks per participant if hCLS-B detected, the block with the highest number of hCLS-B was selected for CD68 staining. If no hCLS-B detected, a block with most WAT was selected	Cross-tabulation of hCLS-B vs. CD68+ CLS-B. Chi-squared tests to assess associations between characteristics and CLS-B presence. Wilcoxon rank-sum tests for associations between body size and metabolic factors. Kaplan-Meier plots with log-rank tests and Cox proportional hazard models to summarize distant disease-free and overall survival related to CLS-B.	hCLS-B positively correlated with higher BMI (p = 0.0008). CD68 + CLS-B positively correlated with higher BMI (p = 0.001). CD68 + CLS-B associated with poor distant disease-free with HR = 2.81, 95% CI 1.20–6.57, and overall survival with HR 3.97 (1.66–9.48), while hCLS-B were not associated with either: HR for distant recurrence 0.59 (0.26–1.30); HR for death 1.04 (0.50–2.16).	Differing metabolic responses, and associated prognostic associations, for CD68 + CLS-B and hCLS-B may reflect differences in macrophage maturity and diversity that are independent of BMI, or potentially other factors not investigated here, including the occurrence of cells other than macrophages in the hCLS-B.
Greenlee, 2019 30404870	91 participants included in final analysis Stage 0-III breast cancer, all underwent mastectomy	Participants underwent mastectomy at Columbia University Medical Center in New York City Hispanic/Latina participants	To assess the relationship between breast white adipose tissue inflammation (BWATi) (defined as presence/ density of CLS) and obesity (measured in BMI)	Stained with hematoxylin and eosin (H&E) and for the macrophage marker, CD68	Normal adjacent breast tissue from a quadrant uninvolved by tumor 1 tissue block per patient, 7 sections cut from each block	The trend test of proportions was used to assess the linear trend in BWATi from low to high BMI levels. Correlates of BWATi were examined using Fisher exact test (categorical variables) and F test (continuous variables). Multivariable and multinomial logistic regression models used to investigate factors associated with the presence or severity of BWATi.	Prevalence of BWATi (CLS-B) increased with BMI (24% in normal weight, 34% in overweight, 57% in class I obesity, and 65% in class II–III obesity; Ptrend <0.01). Severe BWATi (>0.27 CLS-B/cm2) was associated with higher BMI (Ptrend = 0.046) and greater adipocyte diameter (P = 0.04).	BWATi was associated with higher BMI in Hispanic/Latina patients with breast cancer, consistent with previously described associations in other populations.

Iyengar, 2015 25720743	237 mastectomy for breast cancer treatment (n=211) mastectomy for breast cancer risk reduction (n=11) varying stages; all underwent mastectomy	had surgery at MSKCC in NYC NR	1. Does menopause status (independent of BMI) contribute to breast WAT inflammation? 2. Confirm correlations between BMI and breast WAT inflammation, and between breast WAT inflammation and adipocyte diameter 3. Is breast WAT inflammation a sentinel for adipose tissue inflammation in other fat depots?	Stained with hematoxylin and eosin (H&E) and for the macrophage marker, CD68	5 paraffin per case 2 sections per block stained (2 micrometers thick, 2 cm in diameter) When fewer than 5 blocks available, additional sections were cut from same block at 50 micrometers apart A total of 5 sections stained with CD68 generated for each case	Primary endpoints: presence or absence of WAT inflammation (CLS-B/ CLS in abdominal WAT), severity of WAT inflammation defined using median CLS-B density Associations between CLS-B presence and clinicopathologic features (including BMI, menopause status, tumor subtype, race, age, etc.) Logistic regression, Fisher's exact test, Kruskal-Wallis, ANOVA, Chi-squared, Wilcoxon rank-sum, and Spearman's correlation all used where appropriate. Kendall's rank correlation coefficient used to quantify strength of correlation between CLS density in breast and CLS density in abdominal WAT	BMI correlated with presence of CLS-B ($P <$ 0.001) in multivariable model. Positive correlation between adipocyte size in the breast and presence/severity of breast WAT inflammation (p=0.63, P<0.001). The postmenopausal state was associated with the presence of breast WAT inflammation (P=0.008) and was associated with more severe inflammation (higher CLS-B density) (P<0.001). A high rate of concordant CLS-B status (+ or -) found between bilateral breasts, and a high rate of concordant CLS status between breast and abdominal subcutaneous WAT were observed.	Postmenopausal status is independently associated with breast WAT inflammation (CLS-B density). Increased presence/severity of breast WAT inflammation after menopause provides plausible explanation for the paradoxical observation that hormone-dependent tumors occur with increasing frequency as circulating estrogen levels fall. WAT inflammation is systemic and occurs simultaneously in multiple fat depots (contralateral breast and abdominal subcutaneous tissue).
Iyengar, 2016 26712688	Cohort 1: 100 women who underwent mastectomy for breast cancer risk reduction or treatment Cohort 2: 127 women who underwent mastectomy for stage I-III breast cancer and developed distant metastatic disease within follow-up	had surgery at MSKCC in NYC White, Black, Asian	1. To investigate whether breast WAT inflammation is associated with specific circulating factors as well as clinical features of the metabolic syndrome 2. To explore the prognostic importance of breast WAT inflammation on clinical outcomes	Stained with hematoxylin and eosin (H&E) and for the macrophage marker, CD68	Cohort 1: 5 FFPE blocks uninvolved by tumor per participant, 1 section used per block Cohort 2: FFPE block with the most WAT was selected following H&E review, 5 sections from this block used	Nonparametric Wilcoxon rank-sum tests for continuous variables (difference between CLS+ and CLS-), chi-square and Fisher exact tests for categorical variables, Cox proportional hazards regression for univariate and multivariate analyses for association between CLS and distant recurrence free survival	Cohort 1: Patients with CLS-B had elevated insulin, glucose, leptin, triglycerides, C-reactive protein, and IL6 and lower high-density lipoprotein cholesterol and adiponectin ($P < 0.05$) Cohort 2: CLS-B was associated with hyperlipidemia, hypertension, and diabetes ($P < 0.05$). Compared with patients without breast WAT inflammation, the adjusted HR for dRFS was 1.83 (95% CI, 1.07–3.13) for patients with inflammation	WAT inflammation (presence of CLS-B) helps to explain the relationship between metabolic syndrome and worse breast cancer prognosis

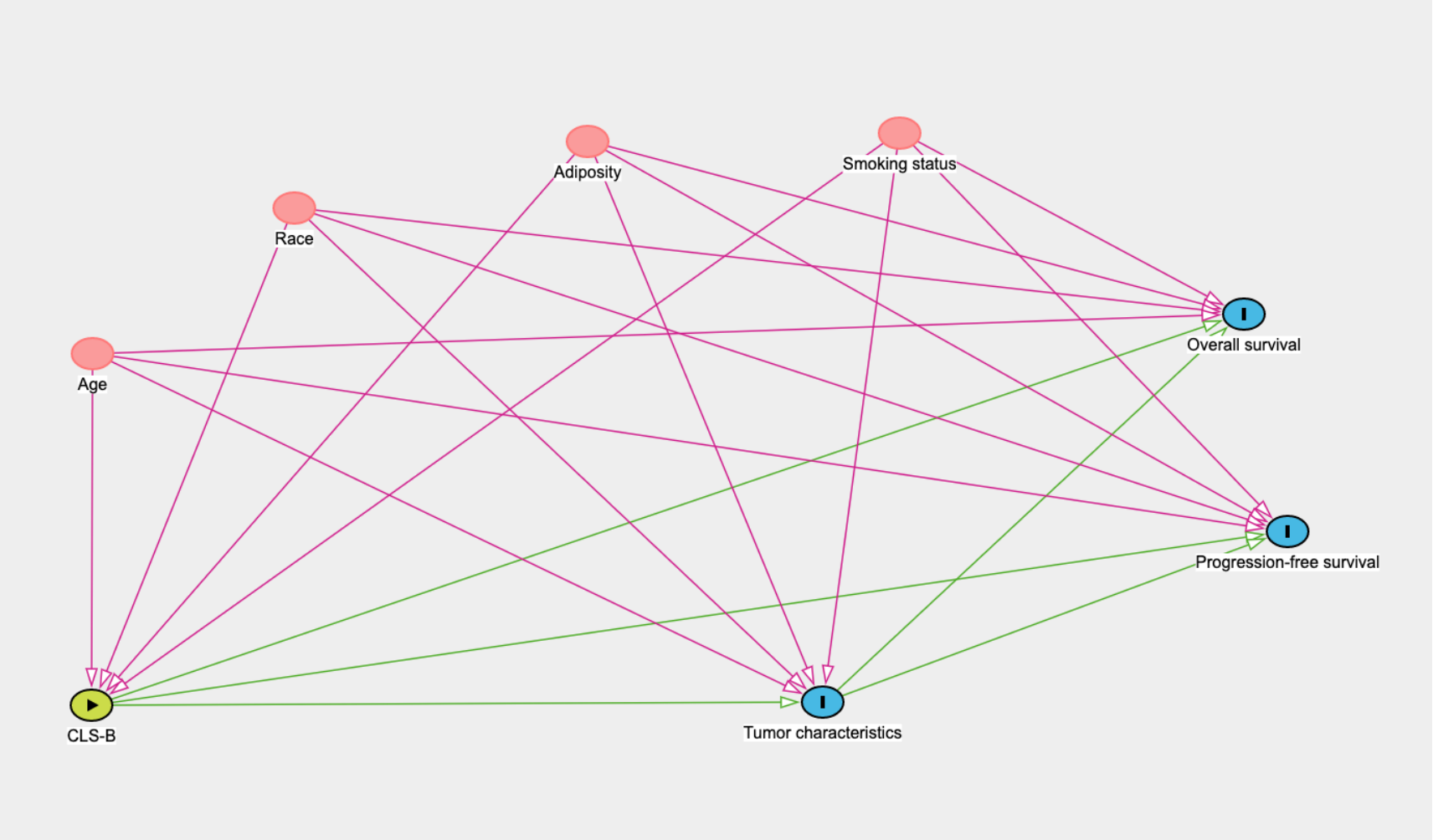
Iyengar, 2017 28270386	72 women with normal BMI (<25 kg/m^2) who underwent mastectomy for breast cancer risk reduction or treatment 15 had noninvasive or benign histology, 57 had either HR+, HER2+, or triple-negative disease	had surgery at MSKCC in NYC White, Black, Asian	1. To determine whether breast WAT inflammation (measured in CLS-B) is associated with aromatase expression and activity among women who underwent mastectomy for breast cancer risk reduction or treatment 2. Assess circulating factors and breast adipocyte size	Stained with hematoxylin and eosin (H&E) and for the macrophage marker, CD68	5 FFPE blocks uninvolved by tumor per participant, 1 section per block used in analysis	Nonparametric Wilcoxon rank-sum tests for continuous variables (difference between CLS+ and CLS-), chi-square and Fisher exact tests used for the categorical severity level of breast WAT inflammation and other categorical variables, Spearman correlation used to examine relationships between 2 continuous variables, Kruskal-Wallis test to examine differences in continuous variables across multiple categories	Median BMI = 23.0 kg/m2 (range, 18.4–24.9 kg/m2) for CLS-B+ versus 21.8 kg/m2 (range, 17.3–24.6 kg/m2) for CLS-B- ($P = 0.04$). CLS-B associated with elevated aromatase expression and activity, which increased with severity of inflammation ($P < 0.05$). Breast WAT inflammation correlated with larger adipocytes ($P = 0.01$) and higher circulating levels of C-reactive protein, leptin, insulin, and triglycerides ($P \leq 0.05$).	A subclinical inflammatory state associated with elevated aromatase in the breast, adipocyte hypertrophy, and systemic metabolic dysfunction occurs in some normal BMI women and may contribute to the pathogenesis of breast cancer
Iyengar, 2018 29222346	Taiwanese cohort: 72 women who underwent mastectomy for breast cancer treatment U.S. Caucasian cohort: 267 women who underwent mastectomy for breast cancer risk reduction or treatment Some with noninvasive or benign histology, some with HR+, HER2+, or triple-negative disease	Had surgery at National Taiwan University Hospital (NTUH, Taipei, Taiwan) or at MSKCC in NYC Taiwanese and Caucasian	1. To examine whether WAT inflammation (CLS-B) and its associated systemic effects correlate with body fat levels in an Asian population where BMI is not an accurate assessment of obesity and cancer risk 2. To investigate whether biologic differences could account for the greater proportion of premenopausal ER+ breast cancer in Asian vs. Western countries	Stained with hematoxylin and eosin (H&E) and for the macrophage marker, CD68	5 tissue sections obtained at 50-micrometer intervals; tissue was uninvolved by tumor	Logistic regression and Fisher exact tests (categorical variables) and nonparametric Wilcoxon rank-sum tests (continuous variables) used to examine the differences between CLS+ and CLS- participants, Spearman correlation used to examine relationships between 2 continuous variables, Kruskal-Wallis test to examine differences in continuous variables across multiple categories, ANOVA and/or t-tests used for associations between mean adipocyte size and categorical variables, Fisher exact and t-tests used to compare variables between the Taiwanese and US cohorts	Taiwanese cohort: CLS-B present in 31 (43%) women and was associated with elevated BMI ($P < 0.01$) and increased levels of body fat ($P < 0.01$), C-reactive protein ($P = 0.02$), triglycerides ($P < 0.01$), insulin resistance scores ($P = 0.04$), and lower HDL cholesterol ($P < 0.01$). ER+ tumors were associated with greater body fat versus other subtypes ($P = 0.03$). Compared with U.S. Caucasian cohort, Taiwanese women had larger breast adipocytes despite lower BMI after adjusting for BMI and menopausal status ($P = 0.01$)	A subclinical inflammatory state associated with increased adiposity and metabolic dysfunction could contribute to breast cancer pathogenesis in Asian women

Koru-Sengul, 2016 27283835	150 women treated for breast cancer between 1978 and 1997, 50 participants in each racial/ethnic group Any stage eligible	Treated at Jackson Memorial Hospital and University of Miami's Sylvester Comprehensive Cancer Center in Miami, FL Black (BL), non-Black Latina (NBLA), Caucasian (CA)	To investigate whether the numbers of CD163+ tumor-associated macrophages (TAMs) and/or CD163+ CLS are associated with patient survival and whether there are significant differences across BL, NBLA, CA	CD163, CD206, CD40, Ki-67	Tumors were FFPE specimens	Differences in means between groups were assessed with Student's t-test or one-way ANOVA with Bonferroni correction for multiple comparisons. Differences in proportions tested using Chi-square or Fisher's exact test. Overall survival and Progression-free survival calculated as elapsed time between the dates of diagnosis and earliest progression (local recurrence, distant metastasis, or death) or last follow-up for patients without progression - Kaplan-Meier curves, and Cox proportional hazard regression models used.	Significant difference in CLS densities ($p = 0.0167$) among BL, NBLA, and CA patients, with BL exhibiting significantly higher densities than CA, and NBLA being in between. The densities of neither CD40 nor CD206 CLS were significantly different among the three racial groups. CLS densities with CD40+ macrophages were significant predictors of OS (HR = 12.15; $p = 0.058$) in univariate analysis, and in bivariate analysis after adjusting the effect of race (HR = 9.14; $p = 0.100$), PR status (HR = 17.43; $p = 0.036$), or HER2 status (HR = 13.59; $p = 0.047$).	Interventions based on targeting TAMs may not only benefit breast cancer patients in general but also serve as an approach to remedy racial disparity resulting in better prognosis patients from minority racial groups
Maliniak, 2020 32552729	174 African American and 168 White women with stage I-III breast cancer treated by mastectomy Stage I-III	Treated at Emory University, or affiliated, hospitals (Atlanta, GA) African American and white women	1. To examine whether patient factors, independent of BMI, are associated with CLS-B in normal adjacent breast WAT tissue of cancer patients 2. To examine whether CLS-B are associated with progression-free and overall survival in a large multi-racial study population	Stained with hematoxylin and eosin (H&E) and for the macrophage marker, CD68	Tissue blocks were distant to the tumor lesion, 1 tissue specimen per participant was used	Logistic regression models used for associations between race, BMI, and other potential factors with the detection of CLS-B. Proportional odds models used to examine associations between potential risk factors and CLS-B density. Survival outcomes by any CLS-B detection were examined using Kaplan-Meier curves and the log-rank test as well as using age-adjusted and multivariable Cox proportional hazards regression.	No observed association between race and CLS-B in multivariable models (OR = 0.82, 95% CI = 0.49–1.36). Detection of CLS-B associated with obesity (OR = 4.73, 95% CI = 2.48–9.01) and age ≥ 60 years at diagnosis (OR = 1.78, 95% CI = 0.99–3.21). Detection of CLS-B was not associated with OS (HR = 1.02, 95% CI = 0.55–1.87) or PFS (HR = 0.99, 95% CI = 0.59–1.67).	There is a strong, positive association between CLS-B and BMI in non-tumor tissue similar to previous findings. Detection of CLS-B did not vary by race and was not associated with worse OS or PFS.

Morris, 2011 21622727	30 varying stages; all underwent mastectomy ipsilateral breast cancer (n=2) contralateral breast cancer (n=14) carcinoma in situ (n=12) no breast cancer hx (n=2)	had surgery at MSKCC in NYC NR	1. Is obesity associated with CLS-B in women? 2. Is obesity-related inflammation associated with increased aromatase expression and activity in the breast?	Stained with hematoxylin and eosin (H&E) and for the macrophage marker, CD68	Ipsilateral invasive tumors (n=2): samples were obtained from quadrants other than the one involved by the tumor Contralateral invasive breast cancer (n=14): no invasive cancer in analyzed breast Carcinoma *in situ*: carcinoma *in situ* only in analyzed breast 5 paraffin per case 2 sections per block stained (2 micrometers thick, 2 cm in diameter)	primary endpoints: CLS-B positivity (presence/absence of CLS-B in any section stained for CD68, CLS-B index used) Associations between CLS-B positivity and baseline patient characteristics were examined using logistic regression and Fisher's exact test (where appropriate) Spearman's rank correlation coefficient used to quantify correlation between CLS-B index and levels of aromatase	14 of 30 (47%) cases had evidence of CLS-B by staining for CD68 - this method was more sensitive than H&E examination of breast WAT (where only n=7 showed evidence of CLS-B) Increasing BMI (normal, overweight, or obese) associated with increased likelihood of CLS-B positivity Increasing BMI associated with increasing CLS-B index in logistic regression ($P < 0.0001$) Adipocyte size associated with increase in CLS-B index ($P = 0.01$)	H&E staining is inadequate for detection of CLS-B. Special stains for CD68 are needed. The presence and severity of CLS-B are associated with increased BMI. A correlation between adipocyte hypertrophy and CLS-B was found. The results support the discovery of the obesity --> inflammation --> aromatase connection that could be used to develop breast cancer risk reduction strategies.
Mullooly, 2017 28103902	83 invasive breast cancers (ER+/PR+ or ER-/PR+)	Patients came from the Polish Breast Cancer Study (PBCS) NR	Whether the identification of CLS is related to concentrations and ratios of sex-steroid hormones in breast adipose tissue relative to systemic circulation	Stained for CD68	Benign tissues were sampled (collected at varying distances from the tumor at the time of surgery)	Mann Whitney U and Kruskal-Wallis analyses assessed relationships between CD68-positive macrophages per unit fat area. Logistic regression used to analyze CLS presence with other clinical characteristics to determine ORs and 95% Cis. Models were adjusted for fat area. Potential effect measure modification of the association between tissue and blood hormone levels with the presence/absence of CLS was examined.	Number of CLS per unit area of fat significantly positively correlated with BMI and age at menopause, inversely correlated with age at first birth ($p < 0.05$ for each correlation). CLS inversely correlated with levels of androstenedione in both tissue and blood ($p < 0.02$ and $p = 0.06$, respectively). Generally null relationships between individual hormone levels and CLS status. Ratios of estrogens to precursor androgens significantly associated with CLS status.	CLS more frequently found among obese women compared to lean women, and therefore might have a greater effect on hormone levels among obese women. Possibility that there are important changes in hormone levels in tissues immediately surrounding CLS which could alter microenvironment. Results do not support evaluating CD68-positive cells as a surrogate of CLS. It remains uncertain whether CLS presence in breast adipose tissue implies information about risk for hormone receptor positive breast cancer beyond that of measuring hormone levels in serum.

Shaik, 2020 33113091	BBD-cancer cohort: 55 women who had BBD that developed into invasive breast cancer BBD only cohort: 47 women with BBD that did not develop into invasive breast cancer KTB cohort: 50 women with no history of BBD nor BrCa Total = 152	BBD-cancer and BBD-only cohorts were based in Detroit, MI KTB cohort from all around the U.S. African American participants only	To examine whether CLS-B and infiltrating lymphocytes (IL) are associated with invasive breast cancer and benign breast disease in African American women	stained for CD68	Single block of FFPE biopsy tissue per participant used	Pearson chi-square and Fisher exact tests for differences in distribution of clinicopathologic characteristics. Kruskal-Wallis or Wilcoxon Signed-Rank test used to test differences in distribution of biopsy population among BMI classes. Linear regression models for associations between adipocyte diameter and clinicopathologic characteristics, multinomial logistic models to estimate odds of being in a specific cohort by CLS-B presence and/or IL	BBD-cancer biopsies were more likely to exhibit CLS-B (OR = 3.36, 95% CI: 1.33–8.48) or IL (OR = 4.95, 95% CI 1.76–13.9) than BBD only biopsies after adjusting for total adipocyte area, adipocyte diameter, proliferative disease, and BMI	CLS-B and IL may serve as histological markers of breast cancer risk in benign breast biopsies from African American women
Vaysse, 2017 28649659	107 women aged 35-75 years Some had conservative surgery (71%), some had mastectomy (29%) histological verified invasive breast cancers stages I-II	Participants came from Energy Balance and Breast Cancer Aspects-II clinical breast cancer study NR	1. Do CLS occur in localized early-stage breast tumors? 2. Is BMI the most appropriate measure of adiposity to reflect the presence of CLS in MAT? 3. Associations among CLS, metabolic dysfunction, and low-grade inflammation. Is MAT inflammation in lumpectomy specimens, as reflected by CLS, associated with mammary adipocyte size, body composition assessed by various methods of assessments, and serum biomarkers, in patients with the most common types of breast tumors in routine clinical practice?	Stained with hematoxylin and eosin (H&E) and for the macrophage marker, CD68. IL6 expression also examined	Used part of the tumor paraffin block that contained the highest amount of surrounding MAT. MAT observed was, therefore, close to the tumor.	T-tests and chi-squared tests used for differences in distribution of characteristics at diagnosis between lean/normal BMI and overweight/obese BMI subgroups. Multivariable logistic regression models used to study associations between anthropometric factors and selected serum markers of CLS. Logistic regression analysis for presence of CLS in overall sample and by menopausal status performed to evaluate various ORs.	Compared with normal BMI participants, overweight and obese patients have 3.2 and 6.9 times OR of CLS, respectively. By e4ach 10% higher truncal fat, a 3.83 higher OR (95% CI 2.07-7.10) of CLS presence in overall population. Positive linear relationship between adipocyte size and CLS density, and a higher adipocyte diameter was observed among CLS-positive patients compared to CLS-negative (P = 0.001).	Presence of CLS in MAT is increased with overweight and obesity. BMI can predict CLS in MAT among postmenopausal women, but measure of truncal fat percentage might be more predictive in premenopausal women. Potential link between visceral adiposity and the presence of CLS in MAT. Association between systemic markers (CRP, TG/HDL cholesterol ratio, glucose, and HbA1c levels) and CLS presence was observed. Excess adiposity, even in overweight patients, is associated with mammary adipose tissue inflammation, an event that could contribute to breast cancer development and progression.

Directed Acyclic Graph (DAG)


Adiposity
Smoking status
Race
Overall survival
Age
Progression-free survival
CLS-B
Tumor characteristics

Supplementary Tables

Supplementary Table 1. Associations of potential risk factors with detection of crown-like structures in breast adipose tissue uninvolved by tumor (CLS-B_{nT}) among 66 women diagnosed with invasive breast cancer (stages I-III), Emory University, 2007-2012

	Any CLS-B_{nT}[a]			
	No N = 46	Yes N = 20	Unadjusted OR[b] (95% CI)	Adjusted OR[b] (95% CI)
Characteristic	**N (%)**	**N (%)**		
Race[c]				
White	29 (63.0)	14 (70.0)	1.00 (-)	1.00 (-)
African American	17 (37.0)	6 (30.0)	0.73 (0.24-2.26)	0.47 (0.14-1.64)
Age at diagnosis (years)[c]				
25 to < 50	19 (41.3)	4 (20.0)	1.00 (-)	1.00 (-)
50 to < 60	11 (23.9)	7 (35.0)	3.02 (0.72-12.7)	3.12 (0.69-14.2)
≥ 60	16 (34.8)	9 (45.0)	2.67 (0.69-10.3)	3.02 (0.73-12.6)
BMI (kg/m^2)[c]				
< 25	25 (54.4)	6 (30.0)	1.00 (-)	1.00 (-)
25 to < 30	9 (19.6)	6 (30.0)	2.78 (0.71-10.9)	3.14 (0.66-15.0)
≥ 30	12 (26.1)	8 (40.0)	2.78 (0.79-9.82)	4.04 (0.89-18.4)
Smoking status[c,g]				
Non-smoker	30 (66.7)	10 (55.6)	1.00 (-)	1.00 (-)
Past smoker	14 (31.1)	7 (38.9)	1.50 (0.47-4.76)	1.56 (0.47-5.24)
Current smoker	1 (2.2)	1 (5.6)	-	-
Age at menarche (years)[c,d]	13.0 (1.7)	12.8 (1.0)	0.94 (0.64-1.38)	1.03 (0.68-1.57)
Parity[c]				
Nulliparous	6 (13.3)	5 (27.8)	1.00 (-)	1.00 (-)
Parous (1+ live births)	39 (86.7)	13 (72.2)	0.40 (0.10-1.53)	0.36 (0.08-1.57)
History of breastfeeding[c,e,g]				
No	11 (30.6)	4 (40.0)	-	-
Yes	25 (69.4)	6 (60.0)	-	-
Menopausal status[c]				
Pre-/perimenopausal	21 (45.7)	6 (33.3)	1.00 (-)	1.00 (-)
Postmenopausal	25 (54.4)	12 (66.7)	0.60 (0.19-1.86)	0.50 (0.08-3.21)
Age at menopause[c,f,g]				
< 45	13 (54.2)	5 (41.7)	-	-
45 to <50	4 (16.7)	4 (33.3)	-	-
50 to < 55	5 (20.8)	2 (16.7)	-	-
≥ 55	2 (8.3)	1 (8.3)	-	-
Hormone replacement therapy use[c,f,g]				
No	13 (54.2)	6 (60.0)	-	-
Yes	11 (45.8)	4 (40.0)	-	-
Family history of breast cancer[c,g]				
No	33 (73.3)	14 (82.4)	-	-
Yes	12 (26.7)	3 (17.7)	-	-
Diabetes mellitus[c,g]				
No	40 (87.0)	19 (95.0)	-	-
Yes	6 (13.0)	1 (5.0)	-	-
Hypertension[c]				
No	28 (60.9)	13 (65.0)	1.00 (-)	1.00 (-)
Yes	18 (39.1)	7 (35.0)	0.84 (0.28-2.50)	0.56 (0.16-2.01)
High cholesterol[c,g]				
No	42 (91.3)	16 (80.0)	-	-
Yes	4 (8.7)	4 (20.0)	-	-

Abbreviations: *BMI* body mass index, *CI* confidence interval, *CLS-B_{nT}* crown-like structures in the breast tissue distant to tumor, *OR* odds ratio

Note: Missing values were not included in the calculation of percentages

[a]Any CLS-B_{nT} was defined as detection of ≥ 1 CLS-B_{nT} with adipocyte encirclement ≥ 50% on the tissue section examined

[b]Estimated odds ratios using logistic regression models with CLS-B_T presence (yes versus no) as the outcome

[c]All models were adjusted for age at diagnosis (years), race (African American, White), and body mass index (kg/m^2) except for those models where these covariates were the exposures of interest in which case covariates were mutually adjusted for; BMI models additionally adjusted for smoking status (never smoker, past smoker, current smoker)
[d]Values expressed as mean (standard deviation)
[e]Among parous women
[f]Among postmenopausal women
[g]No estimated ORs (95% CIs) where data counts < 5 participants

Supplementary Table 2. Associations between detection of crown-like structures in breast adipose tissue uninvolved by tumor (CLS-B_{nT}) and tumor characteristics among 66 women diagnosed with invasive breast cancer (stages I-III), Emory University, 2007-2012

	Any CLS-B_{nT}[a]	
	No (N = 46)	**Yes (N = 20)**
Clinical characteristic		
ER status		
Positive	35 (76.1)	17 (85.0)
Negative	11 (23.9)	3 (15.0)
Stage		
Stage I	25 (54.4)	7 (35.0)
Stage II	18 (39.1)	11 (55.0)
Stage III	3 (6.5)	2 (10.0)
Tumor grade		
Well differentiated	9 (19.6)	3 (15.0)
Moderately differentiated	25 (54.4)	13 (65.0)
Poorly differentiated	12 (26.1)	4 (20.0)
Tumor size (cm)		
< 1 cm	8 (19.5)	2 (10.5)
≥ 1 cm	33 (80.5)	17 (89.5)
Number of positive lymph nodes		
0	31 (70.5)	13 (65.0)
≥ 1	13 (29.6)	7 (35.0)
	Any CLS-B_{nT} vs. None	
	Unadjusted OR (95% CI)	**Adjusted OR (95% CI)**
Outcome		
≥ 1 positive lymph node (vs. 0)	1.28 (0.42-3.95)	1.55 (0.47-5.18)

Abbreviations: *CI* confidence interval, *CLS-B_{nT}* crown-like structures in the breast tissue distant to tumor, *OR* odds ratio
Note: missing values not included in the calculation of percentages
[a]Any CLS-B_{nT} was defined as detection of ≥ 1 CLS-B_{nT} with adipocyte encirclement ≥ 50% on the tissue sectio