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Metabolic syndrome and pre-diabetes contribute to racial disparities in breast cancer outcomes: Hypothesis and Proposed Pathways

Emily J Gallagher¹, Derek LeRoith¹, Rebeca Franco², Irini Markella Antoniou¹, Anupma Nayak³, Jennifer Livaudais-Toman², and Nina A. Bickell^{2,4}

Derek LeRoith: Derek.LeRoith@mssm.edu; Rebeca Franco: Rebeca.Franco@mountsinai.org; Irini Markella Antoniou: irinimarkella.antoniou@mssm.edu; Anupma Nayak: anupma.nayak@mountsinai.org; Jennifer Livaudais-Toman: Jennifer.Toman@ucsf.edu; Nina A. Bickell: Nina.Bickell@mssm.edu

¹Icahn School of Medicine at Mount Sinai, Division of Endocrinology, Diabetes and Bone Disease, Department of Medicine, One Gustave L. Levy Place, Box 1055, New York, NY 10029

²Icahn School of Medicine at Mount Sinai, Department of Population Health Science and Policy, Department of Medicine, One Gustave L. Levy Place, Box 1077, New York, NY 10029

³Icahn School of Medicine at Mount Sinai, Dubin Breast Cancer Center, Department of Pathology, One Gustave L. Levy Place, 1176 Fifth Avenue., New York, NY 10029

⁴Center for Health Equity and Community Engaged Research, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Abstract

Background—Women with obesity and Type 2 diabetes (T2D) are at greater risk of dying from breast cancer than women without these conditions. Obesity and T2D are associated with insulin resistance and endogenous hyperinsulinemia, and are more common in Black women. There is increasing disparity in breast cancer mortality between Black and White women in the U.S. We hypothesize that insulin resistance and endogenous hyperinsulinemia in Black women with breast cancer contribute to their greater breast cancer mortality and are associated with increased insulin receptor signaling in tumors.

Methods—We will recruit 350 Black women and 936 White women with newly diagnosed breast cancer. We will determine the presence or absence of the metabolic syndrome / pre-diabetes, and insulin resistance by measuring body mass index (BMI), waist circumference, lipids, blood pressure, glucose, insulin-like growth factor binding protein 1 and insulin. Breast cancer prognosis will be determined by a Nottingham Prognostic Index (NPI), with poor prognosis being defined as NPI >4.4. Tumor insulin receptor signaling will be determined by immunohistochemistry. Insulin receptor subtype expression will be measured using Nanostring. Analysis of these factors will

Corresponding Author: Emily J Gallagher, **Address:** Division of Endocrinology, Diabetes and Bone Disease, Department of Medicine, One Gustave L. Levy Place, Box 1055, New York, NY 10029, USA, **Telephone:** 212-241-1500, **Fax:** 212-241-4218, Emily.gallagher@mssm.edu.

Competing Interests

The authors declare that they have no competing interests

Author Contributions

All authors contributed to the design of the study, preparation, editing and review of the manuscript.

determine whether endogenous hyperinsulinemia is associated with a worse prognosis in Black women than White women, and increased tumor insulin receptor signaling.

Conclusions—The results of this study will determine if the metabolic syndrome and pre-diabetes contribute to racial disparities in breast cancer mortality. It may provide the basis for targeting systemic insulin resistance and/or tumor insulin receptor signaling to reduce racial disparities in breast cancer mortality.

Keywords

Diabetes; Metabolic Syndrome; Breast Cancer; Racial Disparities; Insulin Resistance; Insulin Receptor

Background

The Cancer Prevention Study II found that overweight and obese women had an increased relative risk of breast cancer mortality compared to women of normal weight, defined by body mass index (BMI) [8]. Similarly, women with Type 2 diabetes also have greater breast cancer mortality than non-diabetic women [6, 9]. Further, individuals with the metabolic syndrome, a syndrome associated with abdominal obesity, impaired glucose tolerance, hypertension and dyslipidemia, also have worse breast cancer outcomes [13]. Previous human studies have found that women with insulin resistance and endogenous hyperinsulinemia have a worse prognosis when diagnosed with early stage breast cancer [16]. Furthermore, animal models have also found that hyperinsulinemia is associated with an increase in primary breast cancer growth and metastasis [17, 18]. Chronically elevated insulin levels may lead to activation of the IR, which is expressed on many cancer cells.

The Health, Eating, Activity and Lifestyle (HEAL) study found that Black women with breast cancer had higher insulin resistance scores than White women when measured by the homeostasis model assessment-estimated insulin resistance (HOMA-IR) [19]. The prevalence of insulin resistance and endogenous hyperinsulinemia is greater in Black than White women in the U.S.; however the studies examining circulating insulin levels and breast cancer incidence and recurrence have largely been performed in White populations. Increased IR expression in pathological breast cancer tissues and increased phosphorylation of the IR/IGF-1R has been associated with a worse prognosis in some studies [20]. Certain breast cancer cell lines derived from Black women express higher levels of the IR [21], but it is unknown whether there is higher IR expression in pathological specimens from Black women compared to White women. Therefore, it is important to understand whether endogenous hyperinsulinemia and IR signaling are key factors contributing to the racial disparity in breast cancer mortality between Black and White women. If insulin resistance and hyperinsulinemia are important factors, then strategies to improve the metabolic health of these women and reduce endogenous hyperinsulinemia or the use of adjuvant therapies to target hyperinsulinemia and the IR signaling pathway may improve survival.

Breast cancer is the second leading cause of cancer death in women in the U.S. [1]. Although breast cancer mortality has been declining overall, there is an increasing disparity in the mortality between Black and White women [2]. A number of biologic and socio-

economic factors may contribute to greater mortality in Black women with breast cancer [3]. Mortality in breast cancer is primarily predicted by the characteristics of the cancer, including stage at presentation, and histological findings including hormone receptor (estrogen receptor (ER) and progesterone receptor (PR)) and HER2 status [4, 5]. Patient characteristics including advanced age at the time of diagnosis, overweight or obesity and the presence of co-morbidities, including diabetes, also impact survival [6–9]. Traditional breast cancer risk factors as well as access to breast cancer screening and treatment contribute to some of the survival disparities, but do not provide a complete explanation [10, 11]. Compared to White women with breast cancer, Black women are more likely to have tumor characteristics associated with poor prognosis (as summarized in Figure 1), including more advanced stage at presentation and ER and PR negative cancers[10]. In addition, there are greater rates of obesity and Type 2 diabetes in the Black population, compared with the White population [12]. Until we understand why Black women with breast cancer have a worse prognosis than White women, we will not be able to improve the prognosis of Black women and prolong their survival.

In this study we aim to determine whether metabolic factors associated with obesity, the metabolic syndrome and Type 2 diabetes are more common in Black women with newly diagnosed breast cancer than White women, and whether these metabolic abnormalities are associated with tumor characteristics associated with a poor prognosis and with increased IR and IGF-1R expression and signaling in the tumors.

Methods / Design

This study was approved by the Institutional Review Board of the Icahn School of Medicine at Mount Sinai.

Patient Accrual

Women over the age of 21 years with newly diagnosed breast cancer will be eligible for the study. Women who self-identify as Black or White race will be recruited. Individuals who identify as Hispanic Black will be included, as prognostic differences between non-Hispanic and Hispanic Black women have not been definitively described. However, we will exclude those who identify as Hispanic White, as this group is more likely to have ER/PR negative tumors than non-Hispanic White women (26% vs 19%, respectively) and would potentially attenuate the association between hormone receptor status and race.

At each participating site, all women over the age of 21 years with a primary diagnosis of breast cancer will be identified and consented prior to their surgery. Patients' demographic characteristics, access to care, factors affecting breast cancer prognosis, overall health status and prognosis and factors affecting insulin resistance will be assessed using a validated questionnaire. Women who have undergone bariatric surgery for weight loss, organ transplantation, have Type 1 diabetes or medically treated Type 2 diabetes, have been taking oral glucocorticoid treatment within two weeks of blood draw and tumor resection, have taken neoadjuvant therapies, have end stage renal disease or hepatic cirrhosis, or are receiving neoadjuvant therapy will be excluded as these conditions can affect insulin levels. The study design is summarized in Figure 2.

Anthropometric Data

Participant's height and weight will be recorded using a stadiometer that will be used throughout the study. Body mass index (BMI) will be calculated from weight (kg)/height² (m²). Waist circumference will be measured at the umbilical level and blood pressure will be recorded using standard National Health and Nutrition Examination Study (NHANES) procedures. Waist circumference and blood pressure along with the fasting preserved blood glucose and lipid measurements will be used to define the metabolic syndrome as described in the Laboratory Measurements section.

Laboratory Measurements

Blood will be drawn after an overnight fast for preserved plasma glucose, serum lipids (total cholesterol, low density lipoprotein (LDL) cholesterol, high-density (HDL) cholesterol, triglycerides (TG)), hemoglobin A1c (HbA1c), serum insulin, C-peptide, IGF-1, insulin-like growth factor binding protein-1 (IGFBP-1). Insulin resistance will be calculated from the HOMA-IR ($[\text{fasting plasma glucose (mg/dL)} \times \text{fasting serum insulin (uU/ml)}]/405$) [22], a widely used and validated measure of insulin resistance [23]. Insulin resistance is defined as a HOMA-IR >2.8, the upper quartile of insulin resistance in the U.S. population, reported by the NHANES [24]. We will assess the presence of the metabolic syndrome, defined by the 2009 International Diabetes Federation (IDF), National Heart, Lung and Blood Institute (NHLBI), American Heart Association (AHA), World Heart Federation (WHF), International Atherosclerosis Society (IAS) and International Association for the Study of Obesity (IASO) joint position statement, as the presence of 3 or more of the following features: waist circumference $\geq 88\text{cm}$; triglycerides (TG) $\geq 150\text{mg/dL}$; high density lipoprotein (HDL) cholesterol $<50\text{mg/dL}$; systolic blood pressure $\geq 130\text{mmHg}$ or diastolic blood pressure $\geq 85\text{mmHg}$; fasting glucose $\geq 100\text{mg/dL}$ or the use of medication to treat elevated TG, low HDL or blood pressure [25].

Determining Prognosis

The final pathology report will be obtained which contains the tumor size, histological grade, nodal status, ER, PR and HER2/Neu status. Where available, Oncotype Dx reports will be recorded. Pathological data is used to calculate the Nottingham Prognostic Index (NPI). The NPI score is calculated as $[0.2 \times \text{tumor size(cm)} + \text{lymph-node stage (1, node negative; 2, 1-3 positive lymph nodes; 3, } \geq 4 \text{ positive lymph nodes)} + \text{histological grade (1, well differentiated; 2, moderately differentiated; 3, poorly differentiated)}]$ [26]. The NPI has maximal prognostic benefit in ER positive, early stage disease, as it measures the intrinsic biological features of a tumor and its probability of metastasizing [27]. Tumor grade will be defined by the Nottingham combined histological grade (NCHG), as recommended by the American Joint Committee on Cancer (AJCC) [5]. Based on a recent U.S. study that dichotomized NPI at 4.4 (poor prognosis >4.4 and good prognosis ≤ 4.4) and showed significant differences in biomarkers (e.g., hormone receptor, Her2) in these groups [28, 29], we will define good prognosis as those women with an NPI score of ≤ 4.4 , and poor prognosis as an NPI score >4.4 . The improved NPI (iNPI) has been suggested to be an even stronger predictor of prognosis than the NPI. The iNPI is calculated by adding 1 point to the NPI for HER2 positivity and subtracting 1 point for PR positivity, with a score of >5.4

signifying a poor prognosis [30]. Therefore, we will determine whether insulin resistance is associated with a poor prognosis, defined primarily by NPI >4.4, and secondarily by iNPI. Because the NPI and iNPI components ER/PR and HER2 status are proven to predict breast cancer survival, these indices will be used as proxy measures of survival to assess the relationship of insulin resistance, metabolic factors and tumor insulin receptors and signaling pathway on poor cancer prognosis.

Immunohistochemical Analysis of Formalin Fixed Paraffin Embedded Tissue Sections

All recruitment sites will fix tissue specimens in 10% formalin for 48–72 hours before processing and embedding in paraffin. 5µm thick sections will be obtained for each patient's tumor paraffin block that was used by the hospital's pathology department to determine tumor ER/PR/HER2 status to maximize the sample's tumor tissue. Immunohistochemistry staining will be performed using previously described methods to detect the presence of the total IRβ (Figure 3), IGF-1Rβ, phosphorylation Akt(Ser473) (Figure 4) and phosphorylation of ERK1/2(Thr202/Tyr204) in the tumor. Staining will be assessed by an Olympus AX70 microscope and automated CellSens imaging analysis software (Olympus, Center Valley, PA, USA) to determine the percent of positive cells in tumor regions of the slide and the intensity of staining, with random samples also assessed by an investigator, blinded to the clinical information of the patient from whom the sample was obtained. The prevalence of IRβ and IGF-1Rβ positive cells, the prevalence of the phosphorylated receptors (pIGF-1R/pIR), Akt phosphorylation (pAkt) and Erk1/2 phosphorylation (pErk1/2) between the two breast cancer prognosis groups will be assessed. For these intracellular markers, the Allred staining system will be used. The Allred score involves assessment of the proportion of positive stained cells and the intensity of staining. The proportion of positive stained cells is rated as 0 = no cells stained positive, 1 = between 0% and 1% stained positive, 2 = between 1% and 10% stained positive, 3 = between 10% and 30% stained positive, 4 = between 33% and 66% stained positive and 5 = between 66% and 100% stained positive. The intensity score is made based on the average intensity of staining, with 0 = negative, 1 = weak, 2 = intermediate and 3 = strong. The intensity score and proportion score will be added together to obtain the Allred score. A score of 0 or 1 will be interpreted as negative [31, 32].

RNA Isolation and Gene Expression Analysis

Four 5µm sections will be cut from formalin fixed paraffin embedded blocks and RNA will be isolated from the samples using the RNeasy FFPE Kit (Qiagen, Valencia, CA), as per the manufacturer's protocol. RNA concentrations will be measured using the Nanodrop ND-1000 (Thermo Scientific, Wilmington, DE). A Custom CodeSet of genes has been designed and generated by Nanostring Technologies (Seattle, WA) to examine the expression of Insulin Receptor (IR) and the Insulin Receptor Isoforms, IR-A and IR-B (Figure 5), in addition to related splicing factors and the proliferation gene Ki67. 150ng of RNA from each sample is subjected to gene expression analysis using Nanostring nCounter at the Mount Sinai qPCR Core Facility. The results will be normalized and analyzed using the nSolver Analysis Software 2.5. Differences in the expression of IR and the expression ratio of IR-A and IR-B will be compared between the White and Black patients, and correlated with laboratory and anthropometric measurements of obesity, the metabolic syndrome, insulin resistance and pre-diabetes.

Outcomes and Statistical Analysis

We will use the NPI rather than cancer stage at presentation to determine prognosis at the time of tumor resection, as the NPI is less affected by health care access than tumor stage and has been validated in several different countries [33, 28, 34]. To ensure the NPI is accurately portraying recurrence risk, we will conduct a small validation study in Year 4 of the study as the majority of early recurrences occur within the first two years of initial treatment [35]. We will call women recruited in Year 2 to ascertain and compare rates of recurrence between women with $\text{NPI} > 4.4$ vs. $\text{NPI} \leq 4.4$. We will calculate percent agreement, kappa, sensitivity and specificity measures comparing NPI to recurrence in this sample.

Descriptive analyses will be performed to profile Black and White women separately including examination of proportions, means, and medians, as well as estimates of variability including standard errors, ranges, and confidence intervals. Continuous data distributions will be evaluated for appropriateness of scale, and transformations will be used where appropriate. For each racial group, we will compute appropriate summary statistics for women who have good prognosis ($\text{NPI} \leq 4.4$; $\text{iNPI} \leq 5.4$) and poor prognosis ($\text{NPI} > 4.4$; $\text{iNPI} > 5.4$). For each population (White and Black), we will compare good and poor prognosis patients with respect to age at diagnosis, year of diagnosis, income, education, access to care, stage at diagnosis family history of breast cancer, smoking status, BMI, waist circumference, blood pressure, lipid profile, medications, use of estrogen, fasting serum insulin, plasma glucose, HbA1c, C-peptide, IGFBP-1, tumor $\text{IR}\beta$, tumor $\text{IGF-1R}\beta$, pAkt, pErk.

Bivariate associations between high endogenous insulin levels and the presence of metabolic syndrome and obesity in each racial group will be explored to determine whether the association between insulin resistance and tumor prognosis for each racial group is mediated by any of these factors. As lack of access to medical care and lower SES are well known to be associated with poor prognosis in breast cancer, due to later stage at diagnosis, different treatment and follow up, we will assess the relationship between preventative screening receipt, access to care and the NPI.

Multivariable analyses for each racial group will be performed to examine the association between insulin resistance ($\text{HOMA-IR} > 2.8$) and poor tumor prognosis ($\text{NPI} > 4.4$) using logistic regression, adjusting for potential confounding factors and assessing mediating factors for each group. Logistic regression will be used to model a binary outcome (poor/good prognosis) and covariates that are significantly associated with insulin resistance and poor prognosis in the sample will be included in the models, as well as those demonstrated in the literature to be strong confounders or mediators. Using the Wald test to identify significant interaction by race (significance level $p < 0.05$), we will determine whether the association between insulin resistance and poor tumor prognosis differs significantly across racial groups. Further, we will assess whether the association between tumor IR and IGF-1R , pAkt and pERK1/2 correlate with tumor prognosis, and whether the degree of insulin resistance (HOMA-IR), serum insulin concentration, and poor tumor prognosis differ significantly between racial groups. In exploratory analyses, we will also further stratify our analyses according to measures of metabolic syndrome to determine whether the

associations between IR expression and tumor prognosis for each racial group are mediated by any of these factors.

Power Analysis

This study is powered to detect a significant interaction by race of the association between insulin resistance (HOMA-IR) and poor prognosis breast cancer (NPI >4.4). Power calculations considered the following factors: the national U.S. prevalence of HOMA-IR >2.8 among healthy, non-diabetic women is 23% in White women, and 33% in Black women; the prevalence of poor prognosis breast cancer, defined as NPI >4.4, in a large, racially and ethnically diverse Health Maintenance Organization (HMO) population of breast cancer patients is 33% in White women and 44% in Black women (unpublished data from Kaiser Permanente Northern California). The power calculation was based on the NPI rather than the iNPI because there is validating data for the NPI. Based on the power analysis, 936 White women and 350 Black women would need to be recruited over 4 years to detect a significant interaction between insulin resistance and poor prognosis in breast cancer by race.

A prior study examining the relationship between insulin resistance and mortality among a sample of predominantly White women with breast cancer found a hazard ratio=1.6 among women with HOMA-IR scores >2.7 [36]. With a sample size of 936 White women, we will have adequate power to detect a main effect OR >1.6 in White women. As research involving other types of chronic disease has identified differential effect sizes by race, with ratios >1.5 comparing effect sizes between Black and White populations, our study is powered to be able to detect a ratio of similar magnitude (1.5) comparing the strength of association between insulin resistance and poor prognosis breast cancer between Black and White women. Under the specified assumptions, this study will have >80% power to detect a significant interaction ratio of 1.5, comparing the effect size in Black women to the effect size in White women (Type I error =0.05). It is also powered (80%) to detect an interaction of this magnitude under several alternative scenarios, given a variety of different prevalences of insulin resistance (range: 10–50%) and a variety of distributions of poor prognosis (range: 20–50%) for White and Black women.

Discussion

The linked epidemics of obesity and diabetes mellitus that disparately affect Black women, coupled by Black women's greater mortality from breast cancer require exploration of possible mechanistic links. How do obesity, the metabolic syndrome, pre-diabetes, insulin resistance, hyperinsulinemia and insulin signaling in tumors result in more aggressive, poorer prognostic breast cancers? This study anticipates finding that insulin resistance and hyperinsulinemia are associated with poor prognostic indices in Black and White women with these conditions more prevalent in the Black population. We also anticipate finding the presence of the IR and IGF-1R in breast cancer tissue, but do not necessarily expect to see any association between the presence of these receptors and the presence of systemic insulin resistance. Rather, we expect to find a racial difference in ratio of IR-A to IR-B, related to the severity of hyperinsulinemia and insulin resistance, and the prognostic index of the

cancer, as other have reported increased IR-A to IR-B ratio in subtypes of breast cancer with a worse prognosis [37, 38]. In contrast to those studies, we expect to find an association between insulin resistance and the presence of increased IR signaling in the tumors. We expect that the phosphatidylinositol 3-kinase (PI3-K)/Akt pathway, rather than the ERK1/2 pathway will be the main signaling pathway activated by the IR in breast cancers from hyperinsulinemic patients. These results would be in keeping with our previous pre-clinical studies [17, 18]. In addition, we anticipate that there will be differences in the expression of IR-A / IR-B ratios, that will be associated with increased insulin resistance and breast cancer with a worse prognostic index.

There are certain limitations to our study that we recognize. In this proposal, we treat race as more of a biologic than a social construct. We ask patients to self-identify race. We are not conducting genetic tests to determine biologic racial categories due to budgetary constraints and the uncertain utility of ancestry information markers. This study is cross-sectional, not longitudinal though we will assess recurrence rates in an early recruited subgroup of women who will have had up to two years time to develop a recurrence. Our outcome variable, a pathology measure of poor prognosis breast cancer, is a proxy of survival. We do not conduct a complete follow-up survey to ascertain recurrence because: 1) tumor size, nodal status, ER and Her2 status have been validated measures of prognosis in studies ascertaining survival and 2) given an expected 2 year recurrence rate of 12%, we would not have adequate power to revalidate these prognostic indices.

This study will not assess the ability of endogenous hyperinsulinemia to stimulate the development of new incident cancer, but rather its potential mechanistic link to cancer progression and mortality. As our primary focus is on the role of hyperinsulinemia and insulin receptor signaling pathways, we do not include tests of chronic inflammation and adipose tissue factors. However, with the patients' consent, we will bank frozen serum to enable future studies on novel markers in obesity, insulin resistance and cancer.

The novel aspect of this study is that it will explore whether insulin resistance, endogenous hyperinsulinemia and IR signaling differ across racial groups, and whether insulin resistance and increased signaling through the IR in breast cancer cells can explain the differences in the breast cancer prognosis in White and Black women. Results may lead to strategies or treatments to reduce the disparity between these groups. Overall, understanding in which patients insulin resistance, hyperinsulinemia and the insulin signaling pathway is important for tumor growth would allow for targeted therapy in this group and could reconcile the disparities in breast cancer mortality between White and Black women.

A randomized controlled trial (RCT) trial testing metformin treatment for breast cancer in the adjuvant setting is currently underway (NCT01101438, <https://clinicaltrials.gov/ct2/show/NCT01101438> last accessed 4th April 2015). The driving hypothesis is that metformin reduces signaling through the PI3K/Akt signaling pathway, activates 5' adenosine monophosphate activated protein kinase (AMPK) which in turn, inhibits mammalian target of rapamycin (mTOR), a protein activated by the IR signaling pathway that helps control cell division and survival. However, the metformin trial will leave unanswered the relationship between insulin resistance, race and poor prognosis breast cancer for two key

reasons: 1) typically, only 2.5% of patients recruited for clinical trials are Black and 2) based on their target accrual of 3,582, the metformin study will not have sufficient power to ascertain racial differences in the relationship between insulin resistance and poor prognosis breast cancer. In addition, women recruited for the study will have already completed primary cancer treatments which are known to affect insulin resistance. Furthermore, patients who end up in RCTs are highly selected and may not be representative of breast cancer patients in the community.

Ultimately, if we discover in humans that hyperinsulinemia and the IR signaling pathway are of importance in breast cancer prognosis, then in the future, examining breast cancer specimens for activation of the IR signaling pathway would allow for the use of therapy targeting this pathway in patients who are refractory to current treatment.

Conclusions

This study is the first specifically designed to address the role of obesity, the metabolic syndrome, pre-diabetes, and concomitant insulin resistance and hyperinsulinemia in racial disparities in breast cancer prognosis with adequate power to detect an interaction by race of the effect of insulin resistance on breast cancer prognosis. If insulin resistance and hyperinsulinemia can explain the differences in prognosis between Black and White women (beyond any differences in SES, access to medical care and other known risk factors for breast cancer survival), then therapies that decrease insulin resistance and block IR signaling may be used to reduce disparities in breast cancer survival.

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List of Abbreviations

BMI	Body Mass Index
ER	Estrogen Receptor
Erk	Extracellular signal-regulated kinase
HbA1c	Hemoglobin A1c
HER2	Human epidermal growth factor receptor 2
HOMA-IR	Homeostasis model assessment of insulin resistance
ICD-9	International Classification of Diseases 9
IGF-1	Insulin-like growth factor 1
IGF-1R	Insulin-like growth factor 1 receptor
IGFBP-1	Insulin -like growth factor binding protein-1

iNPI	Improved Nottingham Prognostic Index
IR	Insulin Receptor
mTOR	mammalian target of rapamycin
NHANES	National Health and Nutrition Examination Study
NPI	Nottingham Prognostic Index
PR	Progesterone Receptor
T2D	Type 2 diabetes

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Figure 1.

Clinical and Biological Factors Potentially Contributing to the Racial Disparities in Breast Cancer Prognosis in Black and White Women.

* Screening consistent with US Preventive Task Force national guidelines

Figure 2.
Schematic of the study design.

Figure 3.
Insulin Receptor Expression in Breast Cancer
Representative image of immunohistochemical staining for the insulin receptor in invasive human breast cancer. Panel A demonstrates positive membrane staining, Panel B is the negative control for the same panel. Images were photographed at 40X objective.

Figure 4.
Akt phosphorylation in Breast Cancer
Representative image of cytoplasmic pAkt staining of human breast cancer specimen. Image was photographed at 40X objective.

Insulin Receptor A and Insulin Receptor B Expression

Figure 5.
Gene Expression analysis of Insulin Receptor Isoform A and B.
Insulin Receptor isoform A and B gene expression as determined by Nanostring nCounter analysis in initial 12 patient samples demonstrating the variation in IR-A and IR-B ratios between patients.