Original Study

A Noninvasive Blood-based Combinatorial Proteomic Biomarker Assay to Detect Breast Cancer in Women Under the Age of 50 Years

Ana P. Lourenco,[1] Kasey L. Benson,[2] Meredith C. Henderson,[2] Michael Silver,[2] Elias Letsios,[2] Quynh Tran,[2] Kelly J. Gordon,[2] Sherri Borman,[2] Christa Corn,[2] Rao Mulpuri,[2] Wendy Smith,[1] Josie Alpers,[3] Carrie Costantini,[4] Nitin Rohatgi,[5] Rebecca Yang,[6] Ali Haythem,[7] Shah Biren,[7] Michael Morris,[8] Fred Kass,[9] David E. Reese[2]

Abstract

To improve breast cancer diagnosis, 2 prospective clinical trials were conducted to test (n = 351) and validate (n = 210) Videssa Breast. If used in conjunction with imaging, Videssa Breast could have reduced unnecessary biopsies by up to 67%. These results support the joint use of breast imaging and Videssa Breast to better inform clinical decisions for women under age 50.

Background: Despite significant advances in breast imaging, the ability to detect breast cancer (BC) remains a challenge. To address the unmet needs of the current BC detection paradigm, 2 prospective clinical trials were conducted to develop a blood-based combinatorial proteomic biomarker assay (Videssa Breast) to accurately detect BC and reduce false positives (FPs) from suspicious imaging findings. **Patients and Methods:** Provista-001 and Provista-002 (cohort one) enrolled Breast Imaging Reporting and Data System 3 or 4 women aged under 50 years. Serum was evaluated for 11 serum protein biomarkers and 33 tumor-associated autoantibodies. Individual biomarker expression, demographics, and clinical characteristics data from Provista-001 were combined to develop a logistic regression model to detect BC. The performance was tested using Provista-002 cohort one (validation set). **Results:** The training model had a sensitivity and specificity of 92.3% and 85.3% (BC prevalence, 7.7%), respectively. In the validation set (BC prevalence, 2.9%), the sensitivity and specificity were 66.7% and 81.5%, respectively. The negative predictive value was high in both sets (99.3% and 98.8%, respectively). Videssa Breast performance in the combined training and validation set was 99.1% negative predictive value, 87.5% sensitivity, 83.8% specificity, and 25.2% positive predictive value (BC prevalence, 5.87%). Overall, imaging resulted in 341 participants receiving follow-up procedures to detect 30 cancers (90.6% FP rate). Videssa Breast would have recommended 111 participants for follow-up, a 67% reduction in FPs ($P < .00001$). **Conclusions:** Videssa Breast can effectively detect BC when used in conjunction with imaging and can substantially reduce unnecessary medical procedures, as well as provide assurance to women that they likely do not have BC.

Clinical Breast Cancer, Vol. 17, No. 7, 516-25 © 2017 ProvistaDx. Published by Elsevier Inc.

Keywords: Biopsies, Imaging, Liquid biopsy, Serum proteins, Tumor-associated autoantibodies

[1]Brown University and Rhode Island Hospital, Providence, RI
[2]Provista Diagnostics, New York, NY
[3]Avera Cancer Institute, Sioux Falls, SD
[4]Scripps Cancer Clinic, San Diego, CA
[5]Sutter Institute for Medical Research, Sacramento, CA
[6]Lahey Clinic, Lahey Medical Center, Peabody, MA
[7]Henry Ford Hospital and Health Network, Detroit, MI
[8]Banner Research, Phoenix, AZ
[9]Sansum Clinic, Santa Barbara, CA

Submitted: Oct 20, 2016; Revised: Apr 14, 2017; Accepted: May 14, 2017; Epub: May 23, 2017

Address for correspondence: David E. Reese, PhD, Provista Diagnostics, 55 Broad St, 18th Fl, New York, NY 10004
E-mail contact: reesed@provistadx.com

http://dx.doi.org/10.1016/j.clbc.2017.05.004

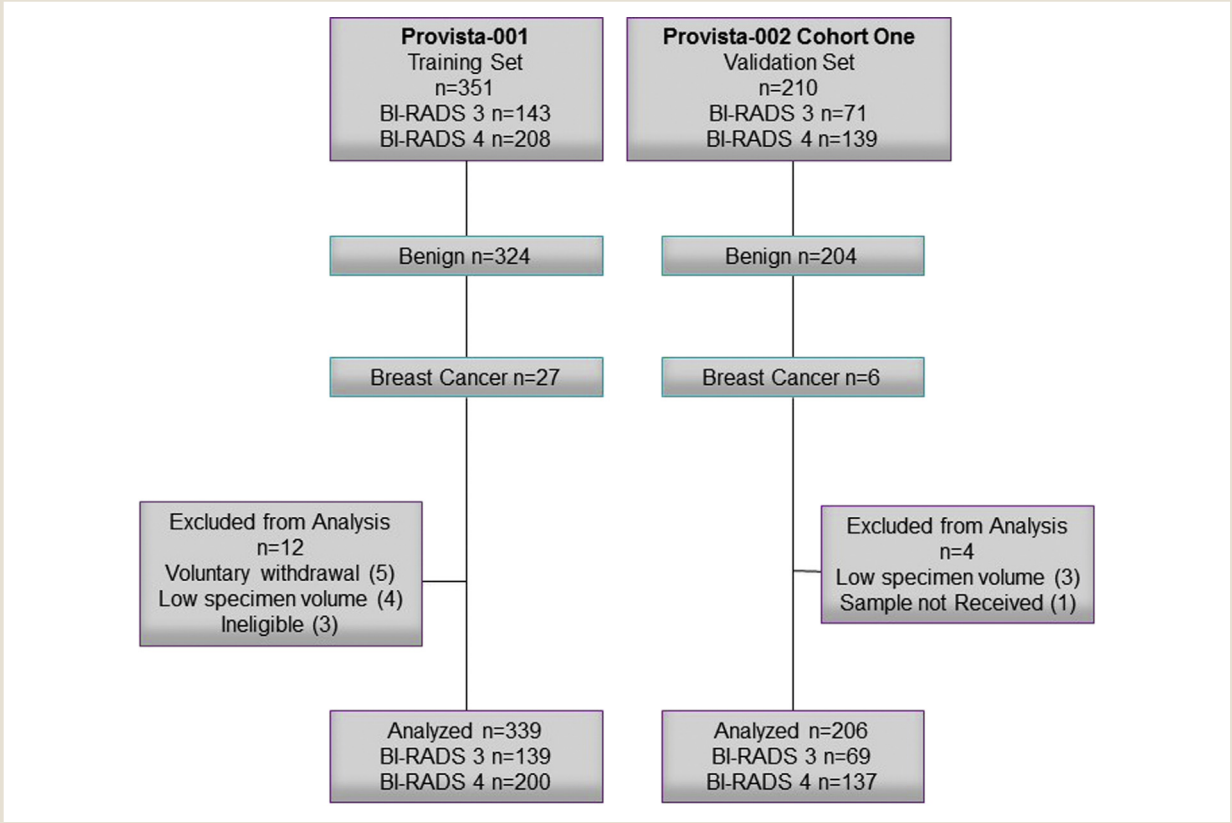
Introduction

Breast cancer (BC) is predicted to be the second leading cause of cancer deaths in women in the United States; approximately 232,000 cases of invasive BC and 60,000 cases of ductal carcinoma in situ (DCIS) are diagnosed and 40,000 deaths occur annually.[1] However, if diagnosed early in a localized state, 5-year survival rates are > 98%.[2]

Imaging (including mammography, ultrasound [US], magnetic resonance imaging [MRI], and 3-D tomosynthesis) is the gold standard for BC detection. It has been suggested that imaging ambiguity could be mitigated by the combination of a proteomic assay.[3] When imaging results are questionable (eg, Breast Imaging-Reporting and Data System [BI-RADS] categories 3 or 4), National Comprehensive Cancer Network guidelines recommend that BI-RADS 3 patients are followed with reimaging at 6 months, and BI-RADS 4 patients are recommended for biopsy.[4] Confounding factors (eg, breast density, prior biopsy, and lesion size) may limit the effectiveness of imaging.[5-7] Women with high breast density have a greater incidence of BC compared with women with low density.[5,8] Despite recent advances in imaging, the rates of false positives (FPs) and false negatives (FNs) represent a significant problem in the early diagnosis of BC.[9-12] The sensitivities and specificities of various imaging modalities and/or their various combinations widely range from 50% upwards.[13-17] A comprehensive study by Berg et al evaluated the supplemental cancer detection yield of US or MRI, when used in addition to mammography, in a large cohort of women (n = 2809; 21 sites) at elevated risk for BC.[15] In this study, sensitivity ranged from 52% upwards and specificity ranged from 65% upwards, depending on the imaging modality used. The addition of screening US or MRI to mammography resulted in more cancers being detected, but there was also an increase in the number of FPs.[15]

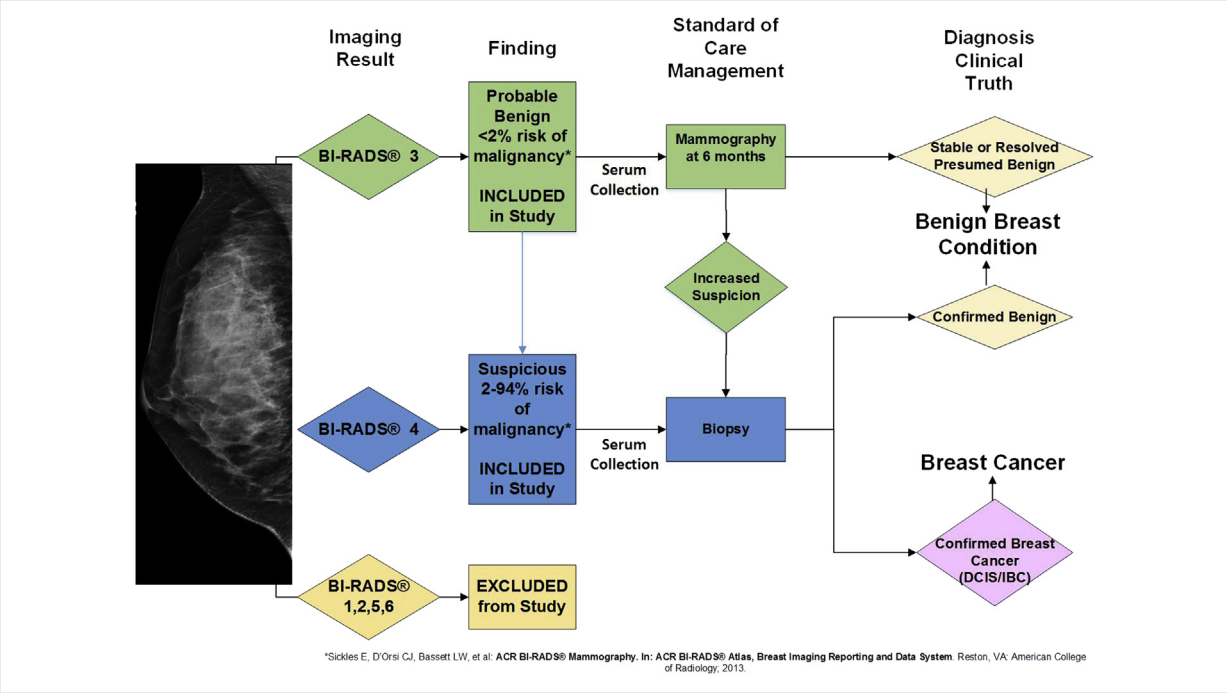
In recent years, the role of protein biomarkers in the detection of BC has undergone a major shift from investigational use to evaluation of prognostic value for a given BC subtype.[11] With the discovery of key protein biomarkers and protein signatures for BC, proteomic technologies are currently poised to serve as an ideal diagnostic adjunct to imaging.[3,18] Previous research studies have shown that breast tumors are associated with systemic changes in both serum protein biomarkers (SPBs) and tumor-associated autoantibodies (TAAbs).[19-27] A very limited number of protein biomarkers, such as the estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2, cancer-associated antigens (CA27.29 and CA15-3), and carcinoembryonic antigen are currently used for prognosis and treatment monitoring, but their utility in detecting early BC has not been confirmed.[28,29] In

Figure 1 Provista-001 and Provista-002 Cohort One (Clinicaltrials.gov Identifiers NCT01839045 and NCT02078570). These Were Prospective Clinical Trials That Enrolled BI-RADS 3 and BI-RADS 4 Patients



Abbreviation: BI-RADS = Breast Imaging Reporting and Data System.

Figure 2 Clinical Management Flowchart. Serum Samples Were Collected From Participants Post-BI-RADS 3 or 4 Assessment Prior to Biopsy in Provista-001 and Provista-002 (Cohort One). BI-RADS 3 and 4 Patients Are Differentially Managed According to Standards of Care, as Summarized in This Flowchart



Abbreviations: BI-RADS = Breast Imaging Reporting and Data System; DCIS = ductal carcinoma in situ; IBC = invasive breast cancer.

addition, several studies have demonstrated the potential use of a new set of protein biomarkers, TAAbs, in early BC detection.[30,31] Given the complexity and heterogeneity of BC, the use of individual protein biomarkers has lacked sensitivity and specificity; a combinatorial biomarker approach may be warranted to ensure the greatest success in detecting BC.[3]

Therefore, to address the unmet needs of the current BC detection paradigm in patients under the age of 50 years assessed as BI-RADS 3 or 4, the aim of this study was to develop a combinatorial proteomic biomarker assay comprised of SPBs and TAAbs, integrated with patient-specific clinical data, to produce a diagnostic score that could reliably detect BC following suspicious imaging findings.

Patients and Methods

Study Design and Participants

Provista-001 (Clinicaltrials.gov, NCT01839045) and Provista-002 (cohort one; Clinicaltrials.gov, NCT02078570), which were sponsored by Provista Diagnostics, enrolled women assessed as either a BI-RADS 3 or 4 at the time of enrollment. All imaging modalities, such as mammography, 3-D tomosynthesis, US, and MRI (and any combination of these modalities) were permitted for the assessment of BI-RADS. Participants were enrolled across 13 domestic clinical sites (See Supplemental Table 1 in the online version), and the study was institutional review board-approved. Informed consent was obtained from all study participants prior to enrollment and sample collection. Blood samples were collected post-imaging and pre-biopsy for all patients enrolled in this study to minimize any potential collection-associated variation. Participants who were not diagnosed with BC were followed for 6 months for additional clinical outcomes, which were assessed via additional imaging and/or pathology results.

Videssa Breast results were not shared with clinicians during the trials to ensure that clinical decision-making was unaffected. An overview of the study design is provided in Figure 1, and the clinical management workflow is summarized in Figure 2. The study was designed by an external subject-matter expert and the authors, and a third-party Contract Research Organization collected and monitored data.

Study Objective

The aim of this study was to develop a blood-based diagnostic test to detect BC for use in conjunction with imaging to aid healthcare providers in making informed decisions on treating young women (under 50 years of age) with difficult-to-assess imaging findings.

Measurement of SPBs and TAAbs

Serum was evaluated for the concentrations of 11 SPBs and for the relative presence/absence of 33 TAAbs (See Supplemental Table 2 in the online version). Following informed consent and prior to biopsy, 5 tubes of blood were collected in a Vacutainer clot tube. Blood was allowed to coagulate for 30 minutes at room temperature, then placed in a centrifuge and spun at 1100× *g* for 10

Table 1 Glossary of Diagnostic Terms Used to Assess Videssa Breast Performance

Sensitivity (True Positives, TP)	**The proportion of subjects with the disease who had a positive test.** Sensitivity = (True Positives) ÷ (True Positives + False Negatives)
Specificity (True Negatives, TN)	**The proportion of subjects without the disease who had a negative test.** Specificity = (True Negatives) ÷ (True Negatives + False Positives)
False Negative (FN) Rate (1 − sensitivity)	**The proportion of subjects with disease but who had a negative test result.** False Negative Rate = (1 − Sensitivity)
False Positive (FP) Rate (1 − specificity)	**The proportion of subjects without disease who had a positive test result.** False Positive Rate = (1 − Specificity)
Positive Predictive Value (PPV)	**The proportion of subjects with a positive test result who actually have the disease.** PPV = (True Positives) ÷ (True Positives + False Positives)
Negative Predictive Value (NPV)	**The proportion of subjects with a negative test result who do not have disease.** NPV = (True Negatives) ÷ (True Negatives + False Negatives)

to 15 minutes. Immediately after centrifugation, a series of aliquots were transferred into 5-mL cryovials, depending on serum yield. Tubes were labeled with a specimen ID number and date, then frozen prior to shipping. Samples were batched and shipped by the site to Provista's laboratory. Upon receipt by Provista, cryovials were accessioned and placed immediately into −80°C for storage.

SPB concentrations were determined using modified electrochemiluminescent-based enzyme-linked immunosorbent assay kits, following manufacturer's specifications (Meso Scale Discovery, Rockville, MD).[32] Each SPB plate contained 6 vendor-provided standards (in duplicate) to generate a standard curve. TAAbs were detected using an indirect enzyme-linked immunosorbent assay, which includes binding purified recombinant proteins to standard-bind plates (Meso Scale Discovery). Proteins were diluted in 1 × phosphate-buffered saline and coated onto blank plates at a final concentration of 20 ng/well. All recombinant proteins, certified as > 80% pure (sodium dodecyl sulfate polyacrylamide gel electrophoresis), were purchased from Origene (Rockville, MD) or Abnova (Taiwan). Origene proteins were myc/DDK peptide-tagged and produced in HEK293 cells. Abnova proteins were glutathione S-transferase-tagged and produced in wheat germ cells.

All samples were processed in duplicate both for SPBs and TAAbs, and mean values were used for data analysis. Appropriate controls (samples with known values, standards, and blanks) were included on each plate to monitor the performance of both assays. SPB concentrations were calculated by processing sample and standard data with the Meso Scale Discovery Workbench 4.0 software using a weighted, 4-parameter, logistic-fit (FourPL) algorithm. TAAb ratio values were determined using the following calculation, using normalization parameters modified from Anderson et al[33]:

(Target MFI − True Target MFI)/Median Sample BKG MFI

where Target MFI = mean fluorescence intensity (MFI) of sample plus target, and True Target MFI = MFI of corresponding target protein without sample (protein background).

Statistical Analysis

Using the Provista-001 dataset only (Figure 1), training models to predict the presence or absence of BC were developed using the individual biomarkers (ie, SPBs and TAAbs). Owing to expectedly weak univariate associations between individual biomarkers based on previous studies,[34,35] additional training models with and without participant's specific clinical data were built iteratively by altering SPB and TAAb features. Multivariable models were built using forward and backward selection methods and varying the alpha for inclusion in (or exclusion from) a model to identify a subset of predictors that consistently presented. These multivariable models were optimized by adding and subtracting additional markers iteratively, until a final model was created that met minimum performance criteria. The area under the receiver operating characteristic was used to determine model performance in regards to sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV); Table 1 provides definitions for these terms. Confidence intervals were reported as 2-sided binomial 95% confidence intervals.

A logistic regression model, which included participant age, was created to combine a panel of transitions that were modeled to calculate a diagnostic score (D_s) between 0 and 1 for each sample, as follows:

$$D_s = 1 \Big/ \left[1 + \exp\left(-\alpha - (\beta_1 * \text{Age}) - \sum_{i=1}^{7} \beta_i * \text{SPB}_i - \sum_{j=1}^{9} \beta_j * \text{TAAb}_j \right) \right]$$

where D_s is diagnostic score, α is intercept, β_1 is coefficient for age, β_i is the coefficient for SPB, and β_j is the coefficient for TAAb. Samples with D_s equal to or greater than the reference value (cutoff) were considered clinically positive (BC), and samples that were less than the reference value were clinically negative (benign).

This training model was tested using the validation set, Provista-002 cohort one (Figure 1). This model was then applied to the combined training (Provista-001) and validation (Provista-002) sets to evaluate its performance in a larger dataset.

Participant characteristics were summarized with medians and inter-quartile ranges for numerical data, or sums and percentages for categorical data. To determine balance between sets, Wilcoxon rank-sum tests were used for continuous variables and χ^2 tests or Fisher

Table 2 Characteristics of Participants Enrolled in Provista-001 and Provista-002 Studies

	Clinical Study				*P* Value
	Provista-001 Training Set		Provista-002 Validation Set		
No. patients	339		206		
Median age, y	43		44		.1248[a]
Age range, y	26-49		26-49		
Race					.01235[b]
Caucasian	266	79%	162	79%	
Black/African American	18	5%	23	11%	
Asian	15	4%	7	3%	
American Indian/Alaska Native/Hawaiian/Pacific Islander	5	2%	5	3%	
Other[c]	35	10%	9	4%	
Ethnicity					.94362[b]
Hispanic or Latino	37	11%	22	11%	
Not Hispanic or Latino[d]	302	89%	184	89%	
BI-RADS category					.07679[b]
3	139	41%	69	33%	
4	200	59%	137	67%	
Biopsies performed	205		136		.20199[b]
BI-RADS 3	15		5		
BI-RADS 4	190		131		
Benign breast condition	313		200		.01865[b]
Pathology confirmed benign	152		121		
Presumed benign[e]	145		76		
Lobular carcinoma in situ[f]	2		2		
Atypical hyperplasia	14		1		
Breast cancer, % incidence	26	7.7%	6	2.9%	.81838[b]
Invasive carcinoma	18		2		
Ductal carcinoma in situ	8		4		

Abbreviation: BI-RADS = Breast Imaging Reporting and Data System.
[a]Statistical significance assessed by the Wilcoxon rank sum test.
[b]Statistical significance assessed by the Fisher exact test.
[c]Multicultural or not reported.
[d]Includes participants that did not report ethnicity.
[e]Presumed all non-cancer participants to be benign.
[f]Lobular carcinoma in situ participants were categorized as non-cancer (benign).

exact tests were used for categorical data, where applicable. All analyses were conducted using SAS (version 9.3; SAS, Cary, NC).

Results

Study Population

The Provista-001 study enrolled 351 women under the age of 50 years at 8 sites (See Supplemental Table 1 in the online version) across the United States, who were assessed as either BI-RADS category 3 or 4 at the time of enrollment. Blood samples were collected post-imaging and pre-biopsy for all patients enrolled in this study to minimize any potential collection-associated variation (Figure 2). Of the 351 participants enrolled, samples collected from 12 participants had to be excluded from analysis (Figure 1), resulting in 339 participants being analyzed for biomarker expression (Table 2). Of these 339 participants, 313 were diagnosed with a benign breast condition, either by biopsy during the initial visit or by additional imaging performed at the 6-month follow-up. Twenty-six participants were diagnosed as having invasive BC (18) or DCIS (8); thus, cancer incidence was 7.7% in Provista-001 (26/339) (Table 2). Of these, 24 participants were diagnosed at the primary visit, and an additional 2 participants were diagnosed during the 6-month follow-up visit.

The Provista-002 study enrolled 210 women under the age of 50 years at 10 sites (See Supplemental Table 1 in the online version), of which 5 overlapped with the Provista-001 study, across the United States who were assessed as either BI-RADS category 3 or 4 at the time of enrollment. Of the 210 participants enrolled, samples collected from 4 participants were excluded from analysis (Figure 1),

leaving 206 participants that could be analyzed for biomarker expression (Table 2). Of these 206 participants, there were 200 diagnosed with a benign breast condition, either by biopsy during the initial visit or by additional imaging performed at the 6-month follow-up visit. Six participants were diagnosed as having BC: BC (2) or DCIS (4). Thus, cancer incidence was 2.9% in the Provista-002 cohort one dataset (6/206) (Table 2). Of these, all participants were diagnosed at the primary visit, and no additional BC cases were diagnosed during the 6-month follow-up visit.

Serum samples were collected post-BI-RADS assessment but prior to biopsy (Figure 2). Samples were evaluated for SPB and TAAb expression as described in the Methods section, and these biomarker expression data were used for Videssa Breast model development.

Videssa Breast Model Development

Previously published results[34] suggested several SPBs (eg, OPN, FASL, TNF-α, carcinoembryonic antigen, IL12, HGF, and VEGFD) and TAAbs (eg, FRS3, RAC3, HOXD1, GPR157, ZMYM6, EIF3E, CSNK1E, ZNF510, BMX, SF3A1, and SOX2) that demonstrated a modest ability to distinguish benign from BC patients. Thus, models were developed using these preselected biomarkers[23,34] (See Supplemental Table 2 in the online version). These preselected markers were univariately evaluated in benign and BC populations; representative box-plots using both age- and BI-RADS-matched samples are provided in Supplemental Figure 1 (in the online version). Because this preselected group of markers was developed utilizing retrospectively collected specimens from 1 group of patients from a single clinical site,[24,34] the inclusion of additional markers was predicted to improve the detection of BC. Additionally, as the prospective cohorts described in this study only included patients with BI-RADS category 3 and 4 diagnoses, these samples represent an intended-use population different than previous studies, which included additional BI-RADS groups 0, 1, 2, 3, 4, and 5 and was not limited to patients aged under 50. The collection of samples from a separate intended-use population further added to the rationale of altering markers to model development.

To develop models that could detect the presence or absence of BC in women under the age of 50 years scored as BI-RADS category 3 or 4, a multi-step process was utilized, whereby biomarker expression and clinical characteristics of the Provista-001 participants (n = 339) were analyzed using logistic regression modeling,[36,37] as described in the Methods.

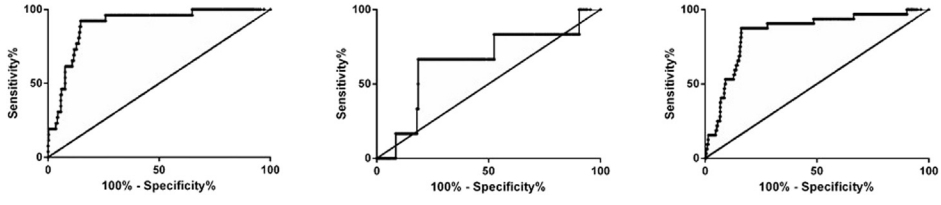
Several training models to detect BC were assessed using different protein biomarker combinations (SPBs and TAAbs) with and without demographics and clinical characteristics, such as age, race, family history, and smoking status (multiple other characteristics were tested but did not show significant differences). Models involving either SPBs or TAAbs alone did not provide statistically significant results (See Supplemental Figure 2 in the online version). The SPB model alone (6 markers) demonstrated high sensitivity (88.5%) and the TAAb model alone (10 markers) demonstrated high specificity (82.5%); these findings confirmed our previous results[34]; therefore, we deduced that combining SPB and TAAb biomarkers would result in a model with higher specificity and higher sensitivity than either biomarker type alone. Starting with our retrospective model,[34] combinatorial training models were built

Figure 3 Clinical Performance of Videssa Breast in Detecting Breast Cancer. A, Performance Data; B, ROC Curves: Provista-001, Provista-002 Cohort One, and Combined Sets

A

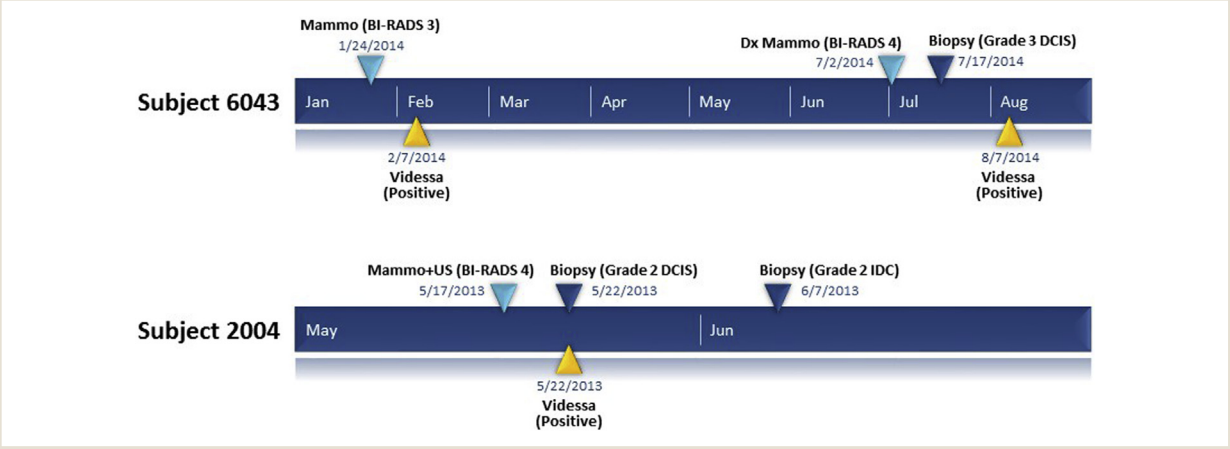
	Observed Breast Cancer Prevalence	Sensitivity	Specificity	PPV	NPV	AUC
Provista-001 (n=339)	7.67%	92.3% *(74.9%, 99.1%)*	85.3% *(80.9%, 89.0%)*	34.3% *(23.3%, 46.6%)*	99.3% *(97.3%, 99.9%)*	0.8995
Provista-002 Cohort One (n=206)	2.91%	66.7% *(22.3%, 95.7%)*	81.5% *(75.4%, 86.6%)*	9.8% *(2.7%, 23.1%)*	98.8% *(95.7%, 99.9%)*	0.6558
Combined (n=545)	***5.87%***	***87.5% (71.0%, 96.5%)***	***83.8% (80.3%, 86.9%)***	***25.2% (17.5%, 34.4%)***	***99.1% (97.7%-99.7%)***	**0.8477**

B



Abbreviations: AUC = area under the curve; NPV = negative predictive value; PPV = positive predictive value; ROC = receiver operator characteristic.

Figure 4 Timeline Progression of 2 Study Subjects. Pertinent Dates Are Shown to Indicate When Imaging Was Performed, When Serum Was Drawn, and When Biopsies Were Performed. Imaging Results Are Shown as BI-RADS Assessments, and Videssa Breast Outcomes Are Provided



Abbreviations: BI-RADS = Breast Imaging Reporting and Data System; DCIS = ductal carcinoma in situ; Dx = diagnosis; IDC = invasive ductal carcinoma; Mammo = mammogram; US = ultrasound.

using both forward and backward selection methods to identify a subset of markers that consistently entered models at various alphas. We then iteratively included or excluded SPB (11) and TAAb (33) markers to optimize sensitivity and specificity while being as parsimonious as possible. No other participant demographic data, except for age, improved the model performance.

The training model consisted of 18 protein biomarkers (8 SPBs and 10 TAAbs) identified from the original set of biomarkers evaluated in this study (See Supplemental Table 2 in the online version). The performance of this model in the training dataset (Provista-001; n = 339) was 92.3% for sensitivity, 85.3% for specificity, 99.3% for NPV, and 34.3% for PPV (Figure 3).

Provista-002 (cohort one; n = 206) used an independent validation set. The training model was locked (ie, biomarker composition, coefficient values, and cut-off point used to detect the presence or absence of cancer) before clinical outcome data for the validation set (Provista-002 cohort one) was received from the blinded data broker. The training model developed using the training set (n = 339) was applied to the validation set (n = 206) to detect BC. A summary of the performance data is provided in Figure 3. The performance of Videssa Breast, when prospectively applied to the validation set (Provista-002 cohort one; n = 206), demonstrated a 66.7% sensitivity, 81.5% specificity, 98.8% NPV, and 9.8% PPV (Figure 3). NPV and specificity are measures of the number of true negative cases within a population. The NPV (98.8%) for Videssa Breast remained extremely high for the validation cohort, which was comparable with the NPV observed for the training set (99.3%; $P = .3023$). The same is true for specificity, which decreased only slightly in the validation cohort (85.3% in training and 81.3% in validation; $P = .24459$).

All benign cases were included in both the training and validation sets, regardless of whether the subject received a biopsy. Of the benign samples collected for this study, 43% were presumed to be benign (ie, no pathologic confirmation by biopsy) (Figure 2), and this included both BI-RADS category 3 and 4 subjects, irrespective of National Comprehensive Cancer Network guidelines, which recommend that BI-RADS 3 patients are followed with reimaging at 6 months, whereas BI-RADS 4 patients are recommended for biopsy.[4] Following model development, performance was specifically evaluated in the confirmed benign (ie, by biopsy) (Figure 2) and BC subgroups (See Supplemental Table 3 in the online version). Sensitivity was unaffected and specificity was increased in both training and validation sets, which could be because of the reduction in FPs (Compare Figure 1 with Supplemental Table 3 [in the online version]). NPV slightly decreased ($P = .82287$) and PPV increased ($P = .00101$) in this subset analysis. These results demonstrate consistent model performance within the intended-use population (where the subject may not undergo biopsy), as well as in the clinically confirmed population (Figure 2).

Owing to low cancer prevalence, both the training (Provista-001) and validation (Provista-002) data sets were combined to assess overall Videssa Breast performance (combined BC prevalence, 5.87%). Videssa Breast correctly diagnosed 28 of the 32 participants with BC (See Supplemental Table 4 in the online version), with a NPV of 99.1%, sensitivity of 87.5%, specificity of 83.8%, PPV of 25.2%, and an area under the curve of 0.8477 (Figure 3). Because of the higher BC prevalence in the training set, it is possible that the sensitivity may suffer from optimism bias; however, specificity and NPV were not impacted. Of note, there were 2 cases that Videssa Breast identified as being positive at enrollment (Sample number 6043 [Figure 4, upper panel] and Sample number 5007 [See Supplemental Table 4 in the online version]), and these cases were not recommended for biopsy after imaging. Subsequent imaging at follow-up recommended these cases for biopsy, and biopsy subsequently confirmed that cancer was present. Thus, Videssa Breast may provide additional diagnostic power to detect early cases of BC.

Detailed timelines for 2 subjects are shown in Figure 4. Subject 6043 was assessed as BI-RADS 3 on initial visit, and no biopsy was

Table 3 Effect of Videssa Breast on Rate of Medical Interventions When Used as an Adjunct to Imaging

	Total Participants Receiving Procedure(s)[a]	TPs	FPs[b]	% Reduction in FPs[c]	*P* Value[d]
Combined				67%	<.0001
Imaging[e]	339	30	309		
BI-RADS 3	19	0	19		
BI-RADS 4	320	30	290		
Videssa Breast	111	28	83		
BI-RADS 3	37	1	36		
BI-RADS 4	74	27	47		

Abbreviations: BI-RADS = Breast Imaging Reporting and Data System; FP = false positive; TP = true positive.
[a]Includes biopsies, cyst aspirations, reduction mammoplasties, lumpectomies, and mastectomies based on enrollment imaging.
[b]Patients who were biopsied not diagnosed with breast cancer on primary visit.
[c]Percent reduction is the reduced number of biopsies that would have been recommended by Videssa Breast compared with standard imaging.
[d]Statistical significance was assessed using the Fisher exact test.
[e]Standard-of-care imaging included diagnostic mammogram, ultrasound, diagnostic mammogram and ultrasound, tomosynthesis, and/or magnetic resonance imaging.

performed. At the 6-month follow-up visit, the subject was assessed as BI-RADS 4, and a subsequent biopsy revealed a high-grade DCIS. Videssa Breast was run on serum drawn at the initial visit and at the 6-month follow-up visit. Both samples resulted in a positive Videssa Breast test result, indicating that this test had correctly identified the subject as likely having BC at the initial visit, when standard assessments failed to detect the presence of BC. Another case, Subject 2004, was assessed as BI-RADS 4 at the initial visit; this subject's Videssa Breast test result was positive. The subject's biopsy revealed a low-grade DCIS; however, an additional biopsy 14 days later revealed a grade 2 BC, indicating that both imaging and Videssa Breast correctly identified the subject as likely having breast adenocarcinoma. Indeed, in this study, when imaging and Videssa concord, 100% detection was observed at the earliest stage.

Comparison of Videssa Breast to Imaging-Based Assessment on Medical Procedure Rate

The imaging modalities used to diagnose BC in participants enrolled in this clinical trial included diagnostic mammogram, US, diagnostic mammogram combined with US, tomosynthesis, and/or MRI. The FP rate (defined as the identification of a benign breast condition by biopsy) of imaging at enrollment was compared with the potential FP rate for Videssa Breast (Table 3). Imaging contributed to 339 participants receiving procedures and detected 30 cancers at enrollment, resulting in 309 FPs (91% FP rate). If Videssa Breast had been used in assessment at the time of enrollment, it would have recommended 111 participants receive procedures, of which 83 would have resulted in a FP (75% FP rate). These data suggest that Videssa Breast, when used in conjunction with imaging, can reduce unnecessary biopsies by up to 67% ($P \leq .00001$) compared with imaging modalities alone.

Discussion

In women with questionable or equivocal imaging findings, it is often difficult to determine whether to proceed with biopsy, further image, or reassess at a later time. Of particular concern is the high FP rate associated with BI-RADS 3 or 4 patients, who are either followed with repeat imaging assessment at 6 months or recommended for biopsy, respectively. The economic impact of FPs is multiplicative owing to the cascade of follow-up diagnostic procedures, such as additional imaging/biopsy, resulting in cumbersome and costly follow-ups. In addition, these follow-up procedures may impact the quality of life for the patient (eg, missing work and family time). Furthermore, scar tissue remaining from biopsy can pose additional complications for future imaging. Perhaps more importantly, the anxiety and negative impact of a positive diagnosis (false or not) on the quality of life for patients is significant and may impact further compliance. Thus, there is a clear need for a diagnostic test that reduces FPs and provides a tool for clinicians to confirm negative findings.

Based on previously published studies suggesting the clinical value of SPBs and TAAbs,[3] we conducted a prospective study to determine if the diagnosis of BC could be improved through the complementary use of a combinational proteomic biomarker assay with imaging. Videssa Breast was successfully developed and validated by combining 8 SPBs and 10 TAAbs with participants' demographic and clinical data; the NPV was 98.8%, sensitivity was 66.7%, specificity was 81.5%, and PPV was 9.8% in the validation set.

We note a decrease in clinical sensitivity between the training and validation sets. Although possibly because of over-fitting of the training model, we feel the bulk of the reduction in Videssa Breast sensitivity in the validation cohort is likely owing to the marked reduced BC prevalence in the Provista-002 cohort as compared with the training set (7.7% vs. 2.9% for Provista-001 and Provista-002, respectively) (Table 2). This reduction may be because of changes in imaging assessments (as a means of decreasing imaging-related FN rates) as this has now been observed in greater than 1350 patients enrolled in Provista's prospective clinical trials over a period of 3 years. Other large studies have also seen a decrease in BI-RADS category 3 use and have witnessed increased BI-RADS category 4 assessment, likely owing to medicolegal considerations. Because sensitivity and PPV were impacted, whereas specificity and NPV were not, it is likely that the decrease in sensitivity and PPV were attributed to low BC prevalence and not over-fitting.

Of the 32 cancers detected by imaging, 2 patients were not recommended for follow-up procedures at enrollment, whereas

Videssa Breast would have recommended these patients for follow-up. Administration of Videssa Breast would have significantly reduced the number of participants receiving procedures prescribed by imaging by 67% ($P < .0001$). Thus, based on these data, Videssa Breast can reduce the number of medical procedures for low- or intermediate-risk BC patients under the age of 50. Conversely, if patients demonstrate positive Videssa Breast results or if additional imaging suggests the presence of BC, further monitoring or biopsy may be warranted.

A significant limitation of this study is that the confirmation of BC through biopsy is subject to sampling bias. A patient can be diagnosed with BC only if the biopsy comes from a region of the breast that includes cells with abnormal lesions. If a biopsy is not performed or is performed in a location absent of neoplasm, BC could be incorrectly diagnosed as a benign breast condition. For example, one of the participants in this study, Subject 1021, was assessed as BI-RADS 4 at the initial visit and underwent a cyst aspiration. Videssa Breast testing on both the initial serum sample and the 6-month follow-up serum sample revealed high levels of p53 TAAb, which is highly indicative of cancer based on previous literature.[38] Upon further review of the patient's medical history, it was noted that this participant had 2 second-degree relatives and 1 first-degree relative diagnosed with BC. There is the possibility that this participant had early BC at the initial visit. When patients have both a positive protein signature for Videssa Breast and a family history of BC, this may warrant additional monitoring—thus, Videssa Breast appears to have utility in aiding physicians to more effectively manage these patients.

Another limitation of this study is that patients were followed for 6 months rather than a 12-month follow-up. A 6-month follow-up period may not be sufficient to identify all cancers in BI-RADS 3 and 4 patients; an additional 12-month follow-up may have yielded an increased cancer incidence. Therefore, it is possible that a subset of the Videssa Breast FPs are pre-clinical BCs that have yet to be detected. This study also relied on multiple imaging modalities to detect BC. The differential diagnostic methodologies used for patients in this clinical trial may have impacted the performance of Videssa Breast, as these methodologies widely vary in their sensitivity and specificity.[15]

An additional study limitation is the BC prevalence in the Provista-002 cohort one set. Whereas a BC prevalence of 20% was expected based on literature,[39] the actual prevalence was 7.7% for Videssa Breast in Provista-001 (training set) and 2.9% for Provista-002 cohort one (validation set). Despite a reduction in sensitivity for the validation set (likely owing to the reduction in BC prevalence from 7.7% to 2.9% for the training and validations sets, respectively), Videssa Breast continued to demonstrate specificity (81.5%) and NPV (99.1%) in this independent validation set. These data provide quantifiable metrics supporting that Videssa Breast can reduce diagnostic uncertainty for providers, based on Videssa Breast specificity, and provide assurance to patients, based on Videssa Breast NPV, that they do not have BC. Thus, Videssa Breast could ultimately reduce the number of unnecessary medical procedures (ie, follow-up imaging and biopsies) and alleviate the stress of not knowing whether an abnormal imaging finding is a BC. Additional studies are being conducted to determine how to best maximize sensitivity (an optimal model for biopsy rule-out) and potential medical cost-savings associated with Videssa Breast in women over age 50.

Conclusion

In summary, this study describes the development of an innovative, noninvasive, actionable tool to detect BC in women under the age of 50. To our knowledge, this is the first prospective study of a proteomic panel (composed of SPBs and TAAbs) being used in the precise detection of BC in woman with questionable imaging findings. Videssa Breast can be used concomitantly with imaging to help guide the management of women under the age of 50 with challenging imaging findings. The test exhibited consistently high specificity and NPV in all test sets in a prospective manner, further supporting its clinical use in this intended-use population (BI-RADS 3 or 4). Further studies evaluating whether Videssa Breast performs similarly in broader age ranges, high-risk populations, and additional BI-RADS-defined patients will be important in assessing the totality of its clinical utility and expanding the clinical use of this personalized, precise, proteomic clinical assay. Additional model development is currently being conducted to maximize sensitivity, thereby increasing clinical utility as a biopsy rule-out test.

Clinical Practice Points

- This study describes the development of an innovative, noninvasive, actionable tool, Videssa Breast, to detect BC in women of low- or intermediate risk (BI-RADS 3 or 4) and under the age of 50.
- To our knowledge, this is the first prospective study of a proteomic panel (composed of SPBs and TAAbs) being used in the precise detection of BC in woman with questionable imaging findings.
- The test exhibited consistently high specificity and NPV in all test sets in a prospective manner, further supporting its clinical use in this intended-use population.
- In women with questionable or equivocal imaging findings, it is often difficult to determine whether to proceed with biopsy, further image, or reassess at a later time. Of particular concern is the high FP rate associated with BI-RADS 3 or 4 patients, who are either followed with repeat imaging assessment at 6 months or recommended for biopsy, respectively. If used prospectively in conjunction with imaging, Videssa Breast could have reduced unnecessary biopsies by up to 67%, compared with standard imaging modalities ($P < .0001$).
- Therefore, this study supports the use of Videssa Breast, concomitantly with imaging, to help guide the management of women under the age of 50 with challenging imaging findings.

Acknowledgments

The authors wish to thank the research staff members at the clinical trial sites for helping conduct the study. The authors would also like to thank all trial participants for their valuable contribution to this work.

This research was funded by Provista Diagnostics.

The Provista-001 and Provista-002 studies both received institutional review board approval prior to initiation. The respective

institutional review boards for each site are provided in Supplemental Table 1 (in the online version). All participants were provided with informed consent and agreed to study participation prior to sample collection.

Disclosure

All authors who are active employees of Provista Diagnostics (K.L.B., M.C.H., M.S., E.L., Q.T., K.J.G., S.B., C.C., R.M., and D.E.R.) own stock of the company. All other authors state that they have no conflicts of interest.

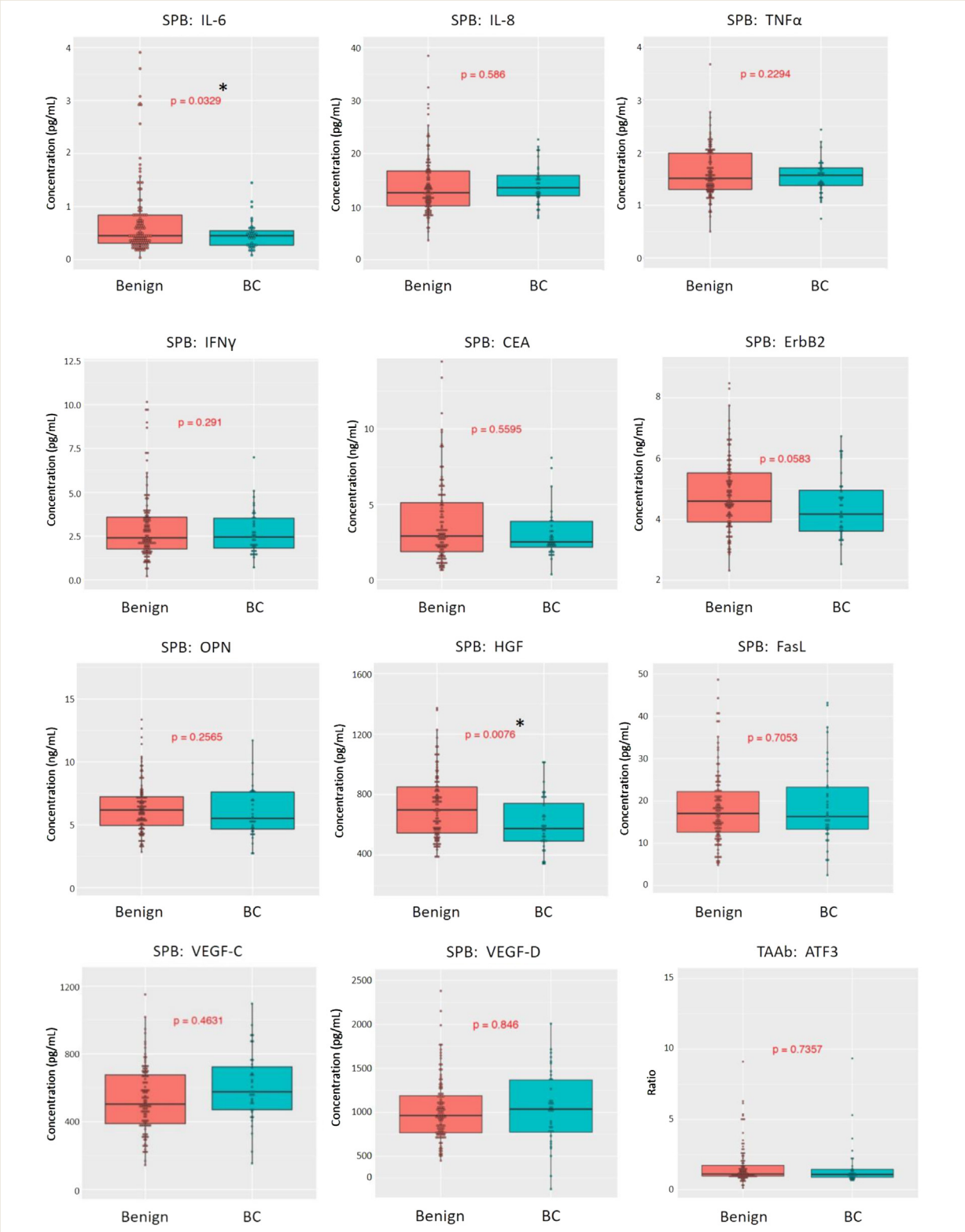
Supplemental Data

Supplemental tables and figures accompanying this article can be found in the online version at http://dx.doi.org/10.1016/j.clbc.2017.05.004.

References

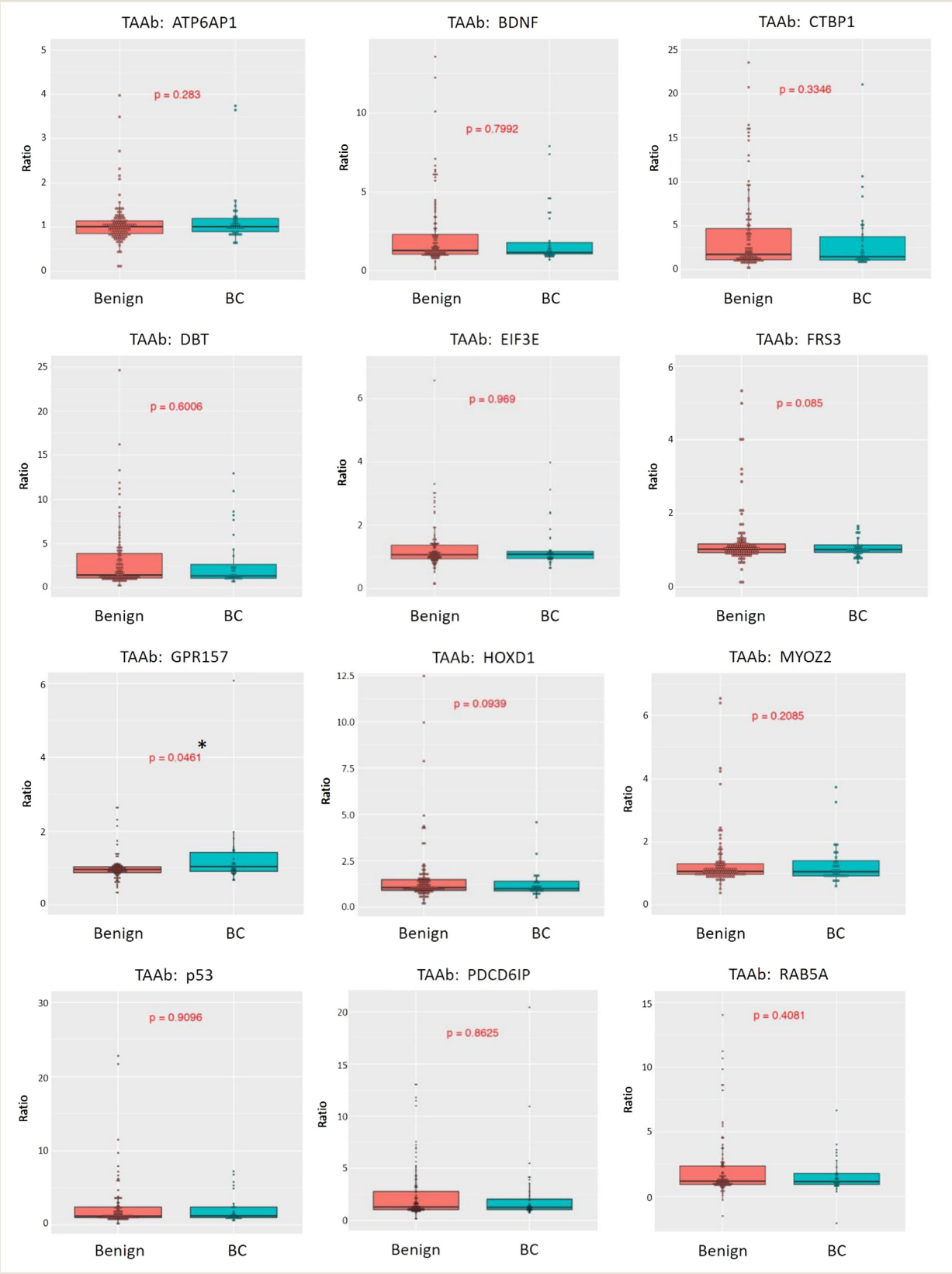
1. *Cancer Facts & Figures, 2015*. Atlanta: American Cancer Society; 2015.
2. *Breast Cancer Facts & Figures, 2011-2012*. Atlanta: American Cancer Society; 2011.
3. Hollingsworth A, Reese D. Potential use of biomarkers to augment clinical decisions for the early detection of breast cancer. *Oncol Hematol Rev* 2014; 10:103-9.
4. Bevers TB, Anderson BO, Bonaccio E, et al, National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology: breast cancer screening and diagnosis. *J Natl Compr Canc Netw* 2009; 7:1060-96.
5. Boyd NF, Guo H, Martin LJ, et al. Mammographic density and the risk and detection of breast cancer. *N Engl J Med* 2007; 356:227-36.
6. Carney PA, Miglioretti DL, Yankaskas BC, et al. Individual and combined effects of age, breast density, and hormone replacement therapy use on the accuracy of screening mammography. *Ann Intern Med* 2003; 138:168-75.
7. van Breest Smallenburg V, Duijm LE, Voogd AC, Jansen FH, Louwman MW. Mammographic changes resulting from benign breast surgery impair breast cancer detection at screening mammography. *Eur J Cancer* 2012; 48:2097-103.
8. Wang AT, Vachon CM, Brandt KR, Ghosh K. Breast density and breast cancer risk: a practical review. *Mayo Clin Proc* 2014; 89:548-57.
9. Pace LE, Keating NL. A systematic assessment of benefits and risks to guide breast cancer screening decisions. *JAMA* 2014; 311:1327-35.
10. Bleyer A, Welch HG. Effect of three decades of screening mammography on breast-cancer incidence. *N Engl J Med* 2012; 367:1998-2005.
11. Cole K, Tabernero M, Anderson KS. Biologic characteristics of premalignant breast disease. *Cancer Biomark* 2010; 9:177-92.
12. Campbell JB. Breast cancer-race, ethnicity, and survival: a literature review. *Breast Cancer Res Treat* 2002; 74:187-92.
13. Kim SA, Chang JM, Cho N, Yi A, Moon WK. Characterization of breast lesions: comparison of digital breast tomosynthesis and ultrasonography. *Korean J Radiol* 2015; 16:229-38.
14. Knuttel FM, Menezes GL, van den Bosch MA, Gilhuijs KG, Peters NH. Current clinical indications for magnetic resonance imaging of the breast. *J Surg Oncol* 2014; 110:26-31.
15. Berg WA, Zhang Z, Lehrer D, et al. Detection of breast cancer with addition of annual screening ultrasound or a single screening MRI to mammography in women with elevated breast cancer risk. *JAMA* 2012; 307:1394-404.
16. Berg WA. Tailored supplemental screening for breast cancer: what now and what next? *AJR Am J Roentgenol* 2009; 192:390-9.
17. Lei J, Yang P, Zhang L, Wang Y, Yang K. Diagnostic accuracy of digital breast tomosynthesis versus digital mammography for benign and malignant lesions in breasts: a meta-analysis. *Eur Radiol* 2014; 24:595-602.
18. *Saving Women's Lives: Strategies for Improving Breast Cancer Detection and Diagnosis*. Washington, DC: The National Academies Press; 2005.
19. Moazzezy N, Farahany TZ, Oloomi M, Bouzari S. Relationship between preoperative serum CA 15-3 and CEA levels and clinicopathological parameters in breast cancer. *Asian Pac J Cancer Prev* 2014; 15:1685-8.
20. Dehqanzada ZA, Storrer CE, Hueman MT, et al. Assessing serum cytokine profiles in breast cancer patients receiving a HER2/neu vaccine using Luminex technology. *Oncol Rep* 2007; 17:687-94.
21. Lyon DE, McCain NL, Walter J, Schubert C. Cytokine comparisons between women with breast cancer and women with a negative breast biopsy. *Nurs Res* 2008; 57:51-8.
22. Rykala J, Przybylowska K, Majsterek I, et al. Angiogenesis markers quantification in breast cancer and their correlation with clinicopathological prognostic variables. *Pathol Oncol Res* 2011; 17:809-17.
23. Anderson KS, Sibani S, Wallstrom G, et al. Protein microarray signature of autoantibody biomarkers for the early detection of breast cancer. *J Proteome Res* 2011; 10:85-96.
24. Benoy IH, Salgado R, Van Dam P, et al. Increased serum interleukin-8 in patients with early and metastatic breast cancer correlates with early dissemination and survival. *Clin Cancer Res* 2004; 10:7157-62.
25. Kovacs E. Investigation of interleukin-6 (IL-6), soluble IL-6 receptor (sIL-6R) and soluble gp130 (sgp130) in sera of cancer patients. *Biomed Pharmacother* 2001; 55:391-6.
26. Kovacs E. The serum levels of IL-12 and IL-16 in cancer patients. Relation to the tumour stage and previous therapy. *Biomed Pharmacother* 2001; 55:111-6.
27. Heer K, Kumar H, Read JR, Fox JN, Monson JR, Kerin MJ. Serum vascular endothelial growth factor in breast cancer: its relation with cancer type and estrogen receptor status. *Clin Cancer Res* 2001; 7:3491-4.
28. Harris L, Fritsche H, Mennel R, et al. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. *J Clin Oncol* 2007; 25:5287-312.
29. Fuzery AK, Levin J, Chan MM, Chan DW. Translation of proteomic biomarkers into FDA approved cancer diagnostics: issues and challenges. *Clin Proteomics* 2013; 10:13.
30. Lacombe J, Mange A, Solassol J. Use of autoantibodies to detect the onset of breast cancer. *J Immunol Res* 2014; 2014:574981.
31. Yahalom G, Weiss D, Novikov I, et al. An antibody-based blood test utilizing a panel of biomarkers as a new method for improved breast cancer diagnosis. *Biomark Cancer* 2013; 5:71-80.
32. Kruse N, Schulz-Schaeffer WJ, Schlossmacher MG, Mollenhauer B. Development of electrochemiluminescence-based singleplex and multiplex assays for the quantification of alpha-synuclein and other proteins in cerebrospinal fluid. *Methods* 2012; 56:514-8.
33. Anderson KS, Cramer DW, Sibani S, et al. Autoantibody signature for the serologic detection of ovarian cancer. *J Proteome Res* 2015; 14:578-86.
34. Henderson MC, Hollingsworth AB, Gordon K, et al. Integration of serum protein biomarker and tumor associated autoantibody expression data increases the ability of a blood-based proteomic assay to identify breast cancer. *PLoS One* 2016; 11:e0157692.
35. Jesneck JL, Mukherjee S, Yurkovetsky Z, et al. Do serum biomarkers really measure breast cancer? *BMC Cancer* 2009; 9:164.
36. Friedman J, Hastie T, Tibshirani R. Additive logistic regression: a statistical view of boosting. *Ann Statist* 2000; 28:337-407.
37. Dettling M, Buhlmann P. Boosting for tumor classification with gene expression data. *Bioinformatics* 2003; 19:1061-9.
38. Soussi T. p53 Antibodies in the sera of patients with various types of cancer: a review. *Cancer Res* 2000; 60:1777-88.
39. Sickles E, D'Orsi CJ, Bassett LW, et al. ACR BI-RADS® Mammography. In: *ACR BI-RADS® Atlas, Breast Imaging Reporting and Data System*. Reston, VA: American College of Radiology; 2013.

Supplemental Figure 1 **Univariate Analysis of Pre-selected Biomarkers. Expression of Individual SPBs and TAAbs (Age- and BI-RADs-matched) Were Evaluated in the Benign and BC Populations. *Denotes Significant Differences in Expression Between Groups**

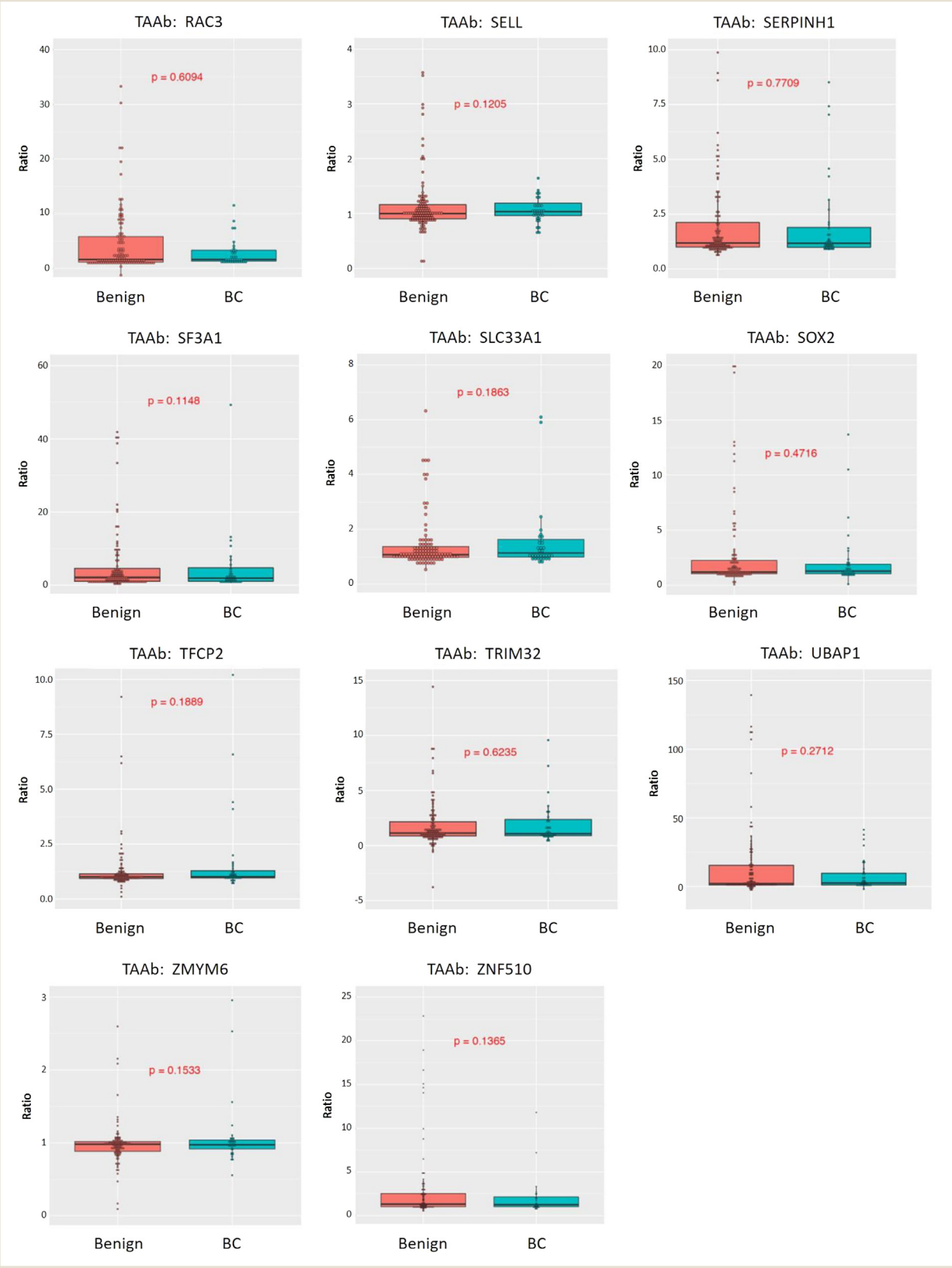


Abbreviations: BC = breast cancer; BI-RADS = Breast Imaging Reporting and Data System; SPB = serum protein biomarker; TAAb = tumor-associated autoantibodies.

Supplemental Figure 1 continued

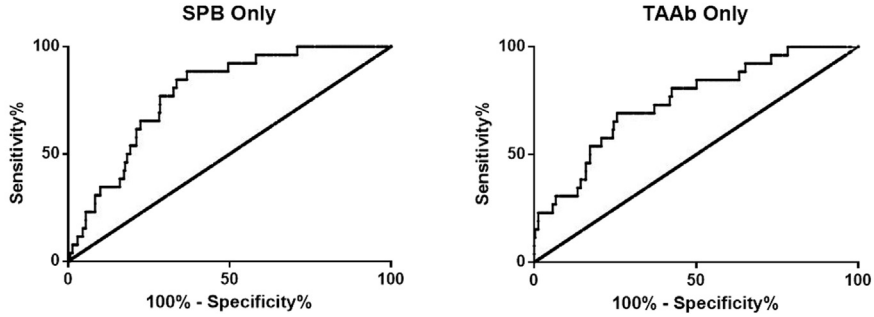


Supplemental Figure 1 continued



Supplemental Figure 2 Performance of SPB and TAAb Independent Models. Training Models Consisting of Only SPBs and Only TAAbs Were Evaluated

Training Model	Sensitivity	Specificity	PPV	NPV	AUC
SPB Only	88.5%	62.9%	16.5%	98.5%	0.7824
TAAb Only	53.8%	82.5%	20.3%	95.6%	0.7476



Abbreviations: AUC = area under the curve; NPV = negative predictive value; PPV = positive predictive value; SPB = serum protein biomarker; TAAb = tumor-associated autoantibodies.

Supplemental Table 1 Enrollment Sites

Clinical Trial	Institution	City	State	IRB
Provista-001/ Provista-002	Avera Cancer Institute	Sioux Falls	SD	Avera Cancer Institute
Provista-001/ Provista-002	Rhode Island Hospital	Providence	RI	Rhode Island Hospital
Provista-001/ Provista-002	Scripps Cancer Clinic	San Diego	CA	Scripps Cancer Center
Provista-001/ Provista-002	Henry Ford Hospital	Detroit	MI	Henry Ford Health System
Provista-001/ Provista-002	Sutter Institute for Medical Research	Sacramento	CA	Chesapeake IRB
Provista-001	Banner Research	Phoenix	AZ	Chesapeake IRB
Provista-001	Lahey Clinic	Peabody	MA	Lahey Hospital Medical Center
Provista-001	Sansum Clinic	Santa Barbara	CA	Chesapeake IRB
Provista-002	Mercy Oncology Center	Oklahoma City	OK	Mercy Health
Provista-002	St. Joseph's Hospital	Phoenix	AZ	Dignity Health St Joseph's
Provista-002	Sinai Grace Detroit Medical Center	Detroit	MI	Western IRB
Provista-002	Mayo Clinic Scottsdale	Scottsdale	AZ	Mayo IRB
Provista-002	Mayo Clinic Rochester	Rochester	MN	Mayo IRB

Abbreviation: IRB = institutional review board.

Supplemental Table 2 SPBs and TAAbs Evaluated During This Study

Protein Class	Protein	Uniprot ID	Full Name From Uniprot	Uniprot Link
SPB	IL-6	P05231	Interleukin-6	http://www.uniprot.org/uniprot/P05231
SPB	IL-8	P10145	Interleukin-8	http://www.uniprot.org/uniprot/P10145
SPB	TNF-α	P01375	Tumor necrosis factor	http://www.uniprot.org/uniprot/P01375
SPB	IFN-γ	P15260	Interferon gamma receptor 1	http://www.uniprot.org/uniprot/P15260
SPB	CEA	P06731	Carcinoembryonic antigen-related cell adhesion molecule 5	http://www.uniprot.org/uniprot/P06731
SPB	ErbB2	P04626	Receptor tyrosine-protein kinase erbB-2	http://www.uniprot.org/uniprot/P04626
SPB	OPN	P10451	Osteopontin	http://www.uniprot.org/uniprot/P10451
SPB	HGF	P08581	Hepatocyte growth factor receptor	http://www.uniprot.org/uniprot/P08581
SPB	FasL	P48023	Tumor necrosis factor ligand superfamily member 6	http://www.uniprot.org/uniprot/P48023
SPB	VEGF-C	P49767	Vascular endothelial growth factor C	http://www.uniprot.org/uniprot/P49767
SPB	VEGF-D	O43915	Vascular endothelial growth factor D	http://www.uniprot.org/uniprot/O43915
TAAb	ALG10	Q5BKT4-1	alpha-1,2-glucosyltransferase	http://www.uniprot.org/uniprot/Q5BKT4
TAAb	ATF3	P18847	Cyclic AMP-dependent transcription factor ATF-3	http://www.uniprot.org/uniprot/P18847
TAAb	ATP6AP1	Q15904	V-type proton ATPase subunit S1	http://www.uniprot.org/uniprot/Q15904
TAAb	BAT4 (GPANK1)	O95872	G patch domain and ankyrin repeat-containing protein 1	http://www.uniprot.org/uniprot/O95872
TAAb	BDNF	P23560	Brain-derived neurotrophic factor	http://www.uniprot.org/uniprot/P23560
TAAb	BMX	P51813	Cytoplasmic tyrosine-protein kinase BMX	http://www.uniprot.org/uniprot/P51813
TAAb	C15orf48 (NMES1)	Q9C002	Normal mucosa of esophagus-specific gene 1 protein	http://www.uniprot.org/uniprot/Q9C002
TAAb	CSNK1E	P49674	Casein kinase I isoform epsilon	http://www.uniprot.org/uniprot/P49674
TAAb	CTAG1A	P78358	Cancer/testis antigen 1	http://www.uniprot.org/uniprot/P78358
TAAb	CTAG2	O75638	Cancer/testis antigen 2	http://www.uniprot.org/uniprot/O75638
TAAb	CTBP1	Q13363	C-terminal-binding protein 1	http://www.uniprot.org/uniprot/Q13363
TAAb	DBT	P11182	Lipoamide acyltransferase component of branched-chain alpha-keto acid dehydrogenase complex, mitochondrial	http://www.uniprot.org/uniprot/P11182
TAAb	EIF3E	P60228	Eukaryotic translation initiation factor 3 subunit E	http://www.uniprot.org/uniprot/P60228
TAAb	FRS3	O43559	Fibroblast growth factor receptor substrate 3	http://www.uniprot.org/uniprot/O43559
TAAb	GPR157	Q5UAW9	Probable G-protein coupled receptor 157	http://www.uniprot.org/uniprot/Q5UAW9
TAAb	HOXD1	Q9GZZ0	Homeobox protein Hox-D1	http://www.uniprot.org/uniprot/Q9GZZ0
TAAb	IGFBP2	P18065	Insulin-like growth factor binding protein 2	http://www.uniprot.org/uniprot/P18065
TAAb	MUC1	P15941	Mucin-1	http://www.uniprot.org/uniprot/P15941
TAAb	MYOZ2	Q9NPC6	Myozenin-2	http://www.uniprot.org/uniprot/Q9NPC6
TAAb	p53	P04637	Cellular tumor antigen p53	http://www.uniprot.org/uniprot/P04637
TAAb	PDCD6IP	Q8WUM4	Programmed cell death 6-interacting protein	http://www.uniprot.org/uniprot/Q8WUM4
TAAb	RAB5A	P20339	Ras-related protein Rab-5A	http://www.uniprot.org/uniprot/P20339
TAAb	RAC3	P60763	Ras-related C3 botulinum toxin substrate 3	http://www.uniprot.org/uniprot/P60763
TAAb	SELL	P14151	L-selectin	http://www.uniprot.org/uniprot/P14151
TAAb	SERPINH1	P50454	Serpin H1	http://www.uniprot.org/uniprot/P50454
TAAb	SF3A1	Q15459	Splicing factor 3A subunit 1	http://www.uniprot.org/uniprot/Q15459
TAAb	SLC33A1	O00400	Acetyl-coenzyme A transporter 1	http://www.uniprot.org/uniprot/O00400
TAAb	SOX2	P48431	Transcription factor SOX-2	http://www.uniprot.org/uniprot/P48431
TAAb	TFCP2	Q12800	Alpha-globin transcription factor CP2	http://www.uniprot.org/uniprot/Q12800
TAAb	TRIM32	Q13049	E3 ubiquitin-protein ligase TRIM32	http://www.uniprot.org/uniprot/Q13049
TAAb	UBAP1	Q9NZ09	Ubiquitin-associated protein 1	http://www.uniprot.org/uniprot/Q9NZ09
TAAb	ZMYM6	O95789	Zinc finger MYM-type protein 6	http://www.uniprot.org/uniprot/O95789
TAAb	ZNF510	Q9Y2H8	Zinc finger protein 510	http://www.uniprot.org/uniprot/Q9Y2H8

Highlighted biomarkers indicate those included in Videssa Breast.
Abbreviations: SPB = serum protein biomarker; TAAb = tumor-associated autoantibody.

Supplemental Table 3 Clinical Performance of Videssa Breast in Subjects Where Benign Diagnosis Was Confirmed by Biopsy

	Cancer, n	Benign, n	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)	AUC
Training	26	168	92.31	87.50	98.66	53.33	0.909
Validation	6	124	66.67	82.26	98.08	15.38	0.669

Abbreviations: AUC = area under the curve; NPV = negative predictive value; PPV = positive predictive value.