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Loss of Breast Cancer Metastasis Suppressor 1 Protein Expression Predicts Reduced Disease-Free Survival in Some Breast Cancer Patients

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Abstract

Purpose—This study aims to determine the effect of loss of *breast cancer metastasis suppressor 1* (BRMS1) protein expression on disease-free survival in breast cancer patients stratified by estrogen receptor (ER), progesterone receptor (PR), or HER2 status, and to determine whether loss of BRMS1 protein expression correlated with genomic copy number changes.

Experimental Design—A tissue microarray immunohistochemical analysis was done on tumors of 238 newly diagnosed breast cancer patients who underwent surgery at the Cleveland Clinic between January 1, 1995 and December 31, 1996, and a comparison was made with 5-year clinical follow-up data. Genomic copy number changes were determined by array-based comparative genomic hybridization in 47 breast cancer cases from this population and compared with BRMS1 staining.

Results—BRMS1 protein expression was lost in nearly 25% of cases. Patients with tumors that were PR negative ($P = 0.006$) or HER2 positive ($P = 0.039$) and <50 years old at diagnosis ($P = 0.02$) were more likely to be BRMS1 negative. No overall correlation between BRMS1 staining and disease-free survival was observed; however, a significant correlation was seen between loss of BRMS1 protein expression and reduced disease-free survival when stratified by either loss of ER ($P = 0.008$) or PR ($P = 0.029$) or HER2 overexpression ($P = 0.026$). Overall, there was poor correlation between BRMS1 protein staining and copy number status.

Conclusions—These data suggest a mechanistic relationship between BRMS1 expression, hormone receptor status, and HER2 growth factor. BRMS1 staining could potentially be used in

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patient stratification in conjunction with other prognostic markers. Further, mechanisms other than genomic deletion account for loss of *BRMS1* gene expression in breast tumors.

The breast cancer metastasis suppressor 1 (*BRMS1*) is one of a growing number of genes that have the ability to suppress metastasis without affecting tumorigenicity in experimental *in vivo* models (1–4). *BRMS1* maps to chromosome 11q13, a region where nonrandom amplification and deletions have been associated with progression and metastasis in breast cancer patients (5). *BRMS1* is a predominantly nuclear protein that contains an imperfect leucine zipper motif and coiled-coiled domains, implying that it may function as part of a transcriptional complex (1), and recent studies suggest that *BRMS1* may inhibit metastasis, in part, through gene regulation via interaction with histone deacetylases (6,7). The restoration of *BRMS1* expression was recently shown to correlate with reduced phosphoinositide and nuclear factor κ B signaling, suggesting specific mechanisms by which *BRMS1* may regulate genes involved in the metastatic process (8,9).

Despite the potential importance of *BRMS1* as a determinant of metastasis in the clinical setting, the study of patient samples from human breast cancer has been hampered by the lack of antibodies to native *BRMS1* (6). The recent development of suitable antibodies to *BRMS1* now makes it possible to study primary breast cancer specimens for protein expression.

In the present study, we examined *BRMS1* expression by immunohistochemistry in a cohort of 238 breast cancer patients with 5-year clinical follow-up, as well as differential genomic gains and losses in a subset of cases from this series using array-based comparative genomic hybridization (aCGH). Our data revealed a strong correlation between loss of *BRMS1* protein expression and reduced disease-free survival in subsets of breast cancer patients when stratified by hormone receptor [estrogen receptor (ER) and progesterone receptor (PR)] or HER2 expression status. Our results also revealed that loss of *BRMS1* protein expression did not correlate with genomic deletion using aCGH, suggesting that mechanisms other than genomic deletion account for loss of *BRMS1* expression in tumors.

Materials and Methods

Patient population and tissue microarrays

A series of previously described and validated tissue microarrays were constructed from tumor tissues of 238 consecutive, newly diagnosed breast cancer patients who underwent surgery at the Cleveland Clinic Foundation between January 1, 1995 and December 31, 1996 (10). The constructed tissue microarray series consisted of a number of 8×12 arrays of 1.5-mm tissue cores from archived formalin-fixed, paraffin-embedded surgical blocks. Two separate tissue cores of invasive carcinoma, totaling a surface area of 3.5 mm^2 , represented each surgical case in the tissue microarray series. This study was approved by the Institutional Review Boards of participating institutions. A unique anonymous identification number was established for each tumor tissue core and linked to an Institutional Review Board – approved database containing 5-year clinical follow-up data.

Immunohistochemistry

BRMS1 expression was assessed by immunohistochemistry of the breast cancer tissue microarray series as well as on a subset (21 of 47) of cases submitted for aCGH using a monoclonal antibody (clone 3a1.21) developed by the University of Alabama, Birmingham Cancer Center Antibody Core Facility using full-length *BRMS1* as an antigen. The specificity of the antibody was confirmed by identification of appropriately sized bands in immunoblots, and by immunoprecipitation, matrix-assisted laser desorption/ionization-time of flight, and electrospray sequencing (electrospray ionization mass spectrometry-mass spectrometry) of immunoprecipitated *BRMS1* to verify that the sequences were *BRMS1* specific (data not

shown). Briefly, 4- μ m-thick unstained sections were placed onto electrostatically charged glass slides and baked overnight. Optimal primary antibody incubation and concentration (1/50) were previously determined via serial dilutions on positive control tissue (placenta). Antigen detection was done with a peroxidase-conjugated secondary antibody/3,3'-diaminobenzidine chromogen step. BRMS1 staining was scored on a 0 to 3+ intensity scale (0, negative nuclear staining; 1+, weak nuclear staining; 2+, moderately intense staining; and 3+, strong staining) by two independent observers blinded to the clinical data, and the results were entered into the research database. Results from the two independent observers were highly concordant with discordant results resolved through agreement. An individual case was considered to be positive if at least one of the two tissue cores contained sufficient tumor for evaluation and at least 10% of invasive tumor cells showed staining. Immunostaining for ER, PR, and HER2 was done as previously published (10).

aCGH

Forty-seven early-stage (I and II) frozen breast tumor samples from 22 patients with early recurrence (<60 months) and 25 age-matched patients who remained disease-free at least 70 months postdiagnosis were analyzed using aCGH. Extracted DNA was quantified and then analyzed on a 5,520 RPCI-11 BAC array containing representative segments from all major chromosome regions (Roswell Park Cancer Institute, Buffalo, NY). Genomic data were standardized and those BAC clones >1.75 or <1.75 of the mean average deviation of the data set were considered as amplified or deleted, respectively.

Statistics

Comparative bivariate statistics was done using χ^2 analysis (BRMS1 staining versus ER staining, PR staining, HER2 status, age >50 years, nodal status, and Bloom-Richardson grade). Survival data were available for 212 of the cases on the tissue microarray and were calculated using the generation of Kaplan-Meier curves. All statistics were carried out using SPSS software (SPSS, Inc., Chicago, IL). Statistical significance was assumed if $P < 0.05$. Mean follow-up of the study population was 67 months (1–106 months).

Results

Loss of BRMS1 expression correlates with earlier age of onset, PR– status, and HER2 overexpression

A summary of the study population is shown in Table 1. The traditional clinicopathologic prognostic variables, including tumor stage, grade, ER/PR status, and HER2 status of the study population, correlated well with survival, as previously published for this patient cohort (10). There were 43.6% stage I, 35.3% stage II, 19.3% stage III, and 1.8% stage IV tumors at the time of diagnosis. Of the 238 breast cancers, 187 (79%) were ER+, 144 (61%) were PR+, and 30 (13%) showed HER2 overexpression by immunohistochemistry.

Normal breast epithelial and myoepithelial cells from control nonneoplastic samples, along with tumor infiltrating lymphocytes, showed strong nuclear immunoreactivity for BRMS1 (2–3+) in all cases (Fig. 1A). In contrast, BRMS1 showed either a diffuse or a granular pattern of nuclear staining within cases of infiltrating breast cancer in both ductal and lobular cell types. BRMS1 immunoreactivity was moderate to strong in 179 (75%) cases (Fig. 1B) whereas it was focally weak to nonimmunoreactive in 59 (25%) cases (Fig. 1C). Among BRMS1-positive cases, nearly all of the infiltrating tumor cells showed strong staining.

The relationship between BRMS1 expression and established clinical and pathologic prognostic factors was examined (Table 2). Patients <50 years old at diagnosis were more likely to be BRMS1 negative ($P = 0.02$), whereas no association was seen between BRMS1

expression and tumor size ($P = 0.34$), grade ($P = 0.13$), or nodal status ($P = 0.5$). BRMS1 expression showed a positive association with PR expression ($P = 0.006$) and a trend towards a positive association with ER, which did not reach statistical significance ($P = 0.09$). An inverse association was also seen between BRMS1 expression and HER2 expression ($P = 0.039$).

Loss of BRMS1 expression correlates with worse disease-free survival in hormone receptor-negative and HER2-overexpressing subgroups of patients

Five-year clinical follow-up data were available for 212 cases enrolled in the study, including time of recurrence and cause of death. For the entire patient population, there was no difference in disease-free and overall survival for BRMS1-negative breast cancer patients compared with BRMS1-positive breast cancer patients ($P = 0.28$; data not shown). Given the association between loss of BRMS1 and PR negative status and between loss of BRMS1 and HER2 overexpression, we looked at patient outcome as a function of expression of BRMS1 and either ER, PR, or HER2 status (Table 3 and Fig. 2A–C, respectively).

Loss of BRMS1 correlated with a significantly worse disease-free survival in ER-negative, PR-negative, and HER2-positive subgroups of cases ($P = 0.008$, $P = 0.029$, and $P = 0.026$; respectively; Fig. 2). Among ER+ breast cancer cases, there was no difference in disease-free survival between BRMS1+ and BRMS1– cases (Fig. 2A). However, in ER– cases, the loss of BRMS1 was associated with a significantly worse disease-free survival (53%) compared with BRMS1+ cases (59%). Similarly, among PR+ cases, stratifying by BRMS1 status did not affect disease-free survival, whereas for PR– cases, loss of BRMS1 was associated with a significantly worse disease-free survival (59%) compared with BRMS+ cases (75%). Similarly, whereas among HER2– cases, no effect on disease-free survival was seen between BRMS1+ and BRMS1– cases (Fig. 2C), in HER2+ cases, there was a significantly worse disease-free survival in BRMS1– (53%) compared with BRMS1+ cases (61%). When looking at ER+/PR– patients, the cumulative disease-free survival was 72.0 months. When stratified by BRMS1 status, ER+/PR–/BRMS1+ patients had a cumulative disease-free survival of 81.8 months, whereas ER+/PR–/BRMS1– patients had a cumulative disease-free survival of 57.8 months (data not shown; $P = 0.171$).

When looking specifically at triple negative (ER–, PR–, and HER–) tumors, we did not observe any effect of loss of BRMS1 expression on cumulative disease-free survival (BRMS1+, 67% disease-free survival; BRMS1–, 60% disease-free survival; $P = 0.607$). However, this subgroup only consisted of 29 patients and the relationship between BRMS1 expression and disease-free survival in cases with triple negative disease remains unclear.

Loss of BRMS1 expression is associated with HER2 protein overexpression

The relationship between hormone receptor status, BRMS1 status, and HER2 status is shown in Table 4. The cases that were ER–/BRMS1– and PR–/BRMS1– showed the highest percentage of tumors that were HER2 positive compared with all of the other subgroups: 5 of 17 (29%) HER2+ of ER–/BRMS1– and 8 of 32 (25%) HER2+ of PR–/BRMS1–, respectively. However, it should be noted that for the ER– group, there was not a substantial difference in HER positivity between the BRMS1+ (9 of 33, 27%) and BRMS1– (5 of 17, 29%) subgroups. For ER+ breast cancers, 9 of 145 (6%) of the BRMS1+ cases were HER2 positive whereas 7 of 42 (17%) of BRMS1– tumors were HER2 positive. For PR+ breast tumors, this trend was also observed, and 7 of 117 (6%) of the BRMS1+ cases were HER2 positive whereas 4 of 27 (15%) of BRMS1– tumors were HER2 positive ($P = 0.01$).

BRMS1 expression is independent of genomic copy number changes on 11q13

Frozen tumor tissue was available on 47 early-stage breast cancer cases from this patient population, which was used to assess genomic copy number changes by aCGH. Of these, 9 were stage I and 38 were stage II. Twenty-five remained disease-free at least 70 months postdiagnosis and 22 had early disease recurrence (<60 months). The majority of cases (32 of 47, 68%) showed euploidy for the BRMS1 region (BAC RP11-80N22) whereas 2 (4%) showed BRMS1 loss and 13 (28%) showed gains of the *BRMS1* gene. There was no significant association between BRMS1 copy number status and disease recurrence in the 47 cases ($P = 0.22$) or by individual stage ($P = 0.23$ for stage I and $P = 0.522$ for stage II).

A subset of the 47 cases (23 of 47) was also analyzed for BRMS1 expression using immunohistochemistry (Table 5). There was moderate to strong nuclear staining (2–3+) in 13 of 23 (56%) of cases and absent to weak staining in 10 of 23 (44%) of cases. There was no statistically significant association overall between BRMS1 protein expression and BRMS1 copy number status ($P = 0.316$). However, note that whereas BRMS1 expression was generally independent of BRMS1 copy number status, the two cases with BRMS1 genomic deletion failed to show any expression of the BRMS1 protein, and six of the seven cases that showed amplification of the BRMS1 were either 2+ or 3+ BRMS1 immunohistochemistry positive. These data imply that genomic status of BRMS1 may influence BRMS1 staining, but that, overall, there is poor correlation between copy number status and BRMS1 staining.

Discussion

We report a significant association between loss of BRMS1 protein expression and worse disease-free survival in subsets of breast cancer patients. Although there was no correlation between loss of BRMS1 staining and cumulative disease-free survival in the entire cohort, when stratified by either loss of ER ($P = 0.008$) or PR ($P = 0.029$) or HER2 overexpression ($P = 0.026$), the BRMS1-negative subgroups had significantly reduced disease-free survival compared with BRMS1-positive cases.

A significant increase in the number of cases staining negative for BRMS1 was also seen in the subgroup diagnosed at age <50 years ($P = 0.02$), suggesting that BRMS1 status may be a more important predictor of clinical outcome in women developing premenopausal breast cancer. Breast cancers that present at a younger age are more likely to be hormone receptor negative, overexpress HER2, and follow a more aggressive clinical course of disease (11–14). Given the fact that loss of BRMS1 in ER-negative, PR-negative, and HER2-positive tumors was associated with the lowest disease-free survival, our data suggest that BRMS1 expression may play a role in mediating the biological and clinical behavior of these subsets of tumors.

Recent reports have suggested that the ER+/PR– phenotype in breast cancer may represent a biologically and clinically distinct subset of tumors compared with ER+/PR+ tumors, both in terms of worse clinical outcome and resistance to antiestrogen therapy (15,16). Tamoxifen-treated patients with ER+/PR– tumors had a significantly worse outcome if their tumors also overexpressed HER1 or HER2, compared with those that showed normal HER1 or HER2 expression. A number of other clinical studies have also reported that HER2 overexpression is associated with decreased benefit from adjuvant tamoxifen treatments (17–19). It has been proposed that PR status may reflect activated HER1/HER2 growth factor signaling in these tumors, and that loss of PR in ER+ breast cancer may represent a surrogate marker for increased growth factor tyrosine kinase activity (15). The correlation between BRMS1 expression and ER and PR expression and the inverse correlation with HER2 expression in the present study suggest a role for BRMS1 in this relationship.

In the current study, those cases that were ER⁻ or PR⁻ were more likely to show HER2 overexpression and a lower disease-free survival, but they were also more likely to show a loss of BRMS1 protein. The PR⁻, HER2⁺ breast cancer phenotype was the most likely to show loss of BRMS1 protein by immunohistochemistry. In fact, examination of cases in the current study showed that even in ER⁺ tumors, the loss of BRMS1 was associated with a higher percentage of cases with HER2 overexpression (BRMS1⁺, 6%; BRMS1⁻, 17%; Table 4). In addition, for both PR⁺ and PR⁻ tumors, the loss of BRMS1 expression was associated with a significantly higher percentage of HER2-overexpressing breast cancer cases (Table 4). Therefore, it is interesting to speculate on a potential role for the loss of BRMS1 expression as a further modulator of the more aggressive clinical behavior of this subset of tumors. These data suggest that BRMS1 expression further stratifies PR⁻ subgroups of breast cancers, possibly in part through regulation of HER family members themselves or through modulation of HER growth factor receptor signaling. However, additional studies will be needed to define the role of BRMS1 in this context.

In this study, loss of BRMS1 expression did not correlate with nodal status. That no correlation was seen may imply that BRMS1 plays a different role in regional and distant metastases. It should be noted that whereas the presence of lymph node metastasis is associated with a worse outcome, this is not a perfect correlation. For example, in a recent study, 29% of patients with >10 positive lymph nodes had not recurred after 10 years of follow-up (20). The likelihood of recurrence correlated with gene expression, but it was unclear if BRMS1 was examined in this study. Thus, among patients with lymph node metastasis, there is biological heterogeneity in terms of who might recur and develop distant metastasis, and the role for BRMS1 in nodal disease remains to be defined.

A recent study examining BRMS1 mRNA expression in human breast cancer clinical samples failed to show any correlation between BRMS1 expression and variables of local dissemination such as tumor size and lymph node metastasis (21). However, in the present study, we found that BRMS1 protein was expressed in all normal mammary epithelia and in unaffected breast tissues from patients with cancer as well as in host inflammatory cells. The immunolocalization of BRMS1 protein was exclusively within the nucleus of both normal and tumor epithelial cells, which is in agreement with the putative role for BRMS1 as a transcription factor (1,6, 7). Therefore, the measurement of BRMS1 mRNA levels in nonmicrodissected clinical samples could potentially be confounded by the expression of the *BRMS1* gene by normal host cells within tumor tissue. Further studies will be needed to clarify this issue.

The mechanism underlying loss of BRMS1 expression in cancer cells is not known. Whereas no mutations in BRMS1 have been reported to date, BRMS1 maps to chromosome 11q13.1–11q13.2, a region that exhibits frequent genomic amplification or deletion associated with breast cancer progression (1). To examine the relationship between genomic copy number changes and BRMS1 protein expression, we did aCGH in a subset of the cases enrolled in this study and compared these data with BRMS1 protein expression. Within this subset of cases, there was some correlation between BRMS1 staining and genomic loss or gain, but for those cases showing no copy number changes, 7 of 14 (50%) showed low to no staining whereas 50% showed high BRMS1 staining. These data imply that genomic status of BRMS1 may influence BRMS1 staining, but that, overall, there is poor correlation between copy number status and BRMS1 staining. These findings suggest that an alternative mechanism, such as epigenetic promoter hypermethylation, may explain loss of BRMS1 protein expression in many of these tumors. Amplification of this region was found in approximately one third of tumors, suggesting that other genes located in proximity to BRMS1 on chromosome 11 may also be involved in breast cancer progression.

A major challenge in the treatment of breast cancer is to identify those patients that are more likely to develop metastases so that appropriate treatment can be provided. Although survival is best correlated with regional lymph node status, other differentially expressed markers in the primary tumor are also imperfectly predictive. In this study, we show that loss of BRMS1 expression is associated with decreased disease-free survival in the context of hormone receptor-negative tumors. We also show a correlation between loss of BRMS1 and HER2 overexpression, suggesting a possible mechanistic relationship between BRMS1 and HER2 growth factor signaling that may have potential implications for therapeutic intervention. These data suggest that BRMS1 staining could potentially be used in conjunction with other prognostic markers for patient stratification.

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Fig. 1.

A, normal breast ductal epithelium and myoepithelial cells showing strong positive (3+) staining for BRMS1 ($\times 200$ magnification). In addition, scattered endothelial and inflammatory cells in the adjacent stroma can be seen showing staining, serving as an internal positive control. B, BRMS1-positive breast cancer case showing strong nuclear immunoreactivity within tumor cells as well as staining of adjacent endothelium ($\times 400$ magnification). C, BRMS1-negative breast cancer case showing a loss of nuclear staining within tumor cells ($\times 400$ magnification).

Fig. 2.

Kaplan-Meier curves showing the disease-free survival for subgroups of patients stratified by ER/BRMS1 staining (A), PR/BRMS1 staining (B), and HER2/BRMS1 staining (C). Loss of BRMS1 expression led to a further reduction in disease-free survival in ER-negative ($P = 0.008$), PR-negative ($P = 0.028$), and HER2-positive ($P = 0.026$) subgroups.

Table 1
Clinical and pathologic characteristics of the study population ($n = 238$ subjects)

Criteria	No. cases (%)
Age (y)	
<50	55 (23)
>50	167 (70)
Unknown	16 (7)
Tumor size	
T1	123 (52)
T2	68 (28)
T3	14 (6)
T4	11 (5)
Unknown	22 (9)
Bloom-Richardson grade *	
1	31 (13)
2	100 (42)
3	64 (27)
Infiltrating lobular/other	43 (18)
Axillary lymph node status	
N ₀	121 (51)
N ₁	53 (22)
N ₂	19 (8)
N ₃	10 (4)
Lymph node dissection not done	17 (7)
Unknown	18 (8)
ER	
Negative	50 (21)
Positive	187 (79)
Unknown	1 (0)
PR	
Negative	93 (39)
Positive	144 (61)
Unknown	1 (0)
HER2 status (immunohistochemistry)	
Negative (0–1+)	208 (87)
Overexpressed (2–3+)	30 (13)
BRMS1	
Negative	59 (25)
Positive	179 (75)

* Infiltrating lobular and rare infiltrating ductal variants not assigned a Bloom-Richardson grade.

Table 2
Clinical and pathologic characteristics of the study population as a function of BRMS1 expression

	BRMS1+ (n= 179)	BRMS1- (n= 59)	P
Age (y)			
<50	35 of 167 (21%)	20 of 55 (36%)	0.02
>50	132 of 167 (79%)	35 of 55 (64%)	
Tumor size			
T1	90 of 160 (56%)	33 of 56 (59%)	0.34
T2	52 of 160 (32.5%)	16 of 56 (28.5%)	
T3	12 of 160 (7.5%)	2 of 56 (3.5%)	
T4	6 of 160 (4%)	5 of 56 (9%)	
Bloom-Richardson grade			
1	27 of 144 (19%)	4 of 51 (8%)	0.13
2	69 of 144 (48%)	31 of 51 (61%)	
3	48 of 144 (33%)	16 of 51 (31%)	
Lymph node status			
N ₀	94 of 151 (62%)	27 of 52 (52%)	0.50
N ₁	37 of 151 (25%)	16 of 52 (31%)	
N ₂	14 of 151 (9%)	5 of 52 (9%)	
N ₃	6 of 151 (4%)	4 of 52 (8%)	
ER			
Negative	33 of 178 (19%)	17 of 59 (29%)	0.09
Positive	145 of 178 (81%)	42 of 59 (71%)	
PR			
Negative	61 of 178 (34%)	32 of 59 (54%)	0.006
Positive	117 of 178 (66%)	27 of 59 (46%)	
HER2			
Negative	161 of 179 (90%)	47 of 59 (80%)	0.039
Positive	18 of 179 (10%)	12 of 59 (20%)	

Table 3

Disease-free survival for breast cancer subgroups

Population subgroup (n)	Cumulative DFS* (mo)
ER+ all (168)	82% DFS (107)
ER+/BRMS1+ (128)	83% DFS (107)
ER+/BRMS1- (40)	80% DFS (88)
ER- all (43)	58% DFS (105)
ER-/BRMS1+ (28)	59% DFS (105)
ER-/BRMS1- (15)[†]	53% DFS (104)
PR+ all (130)	81% DFS (107)
PR+/BRMS1+ (104)	79% DFS (107)
PR+/BRMS1- (26)	88% DFS (87)
PR- all (81)	69% DFS (104)
PR-/BRMS1+ (52)	75% DFS (93)
PR-/BRMS1- (29)	59% DFS (104)
HER2+ all (24)	57% DFS (105)
HER2+/BRMS1+ (13)	61% DFS (105)
HER2+/BRMS1- (11)	53% DFS (88)
HER2- all (188)	79% DFS (107)
HER2-/BRMS1+ (144)	80% DFS (107)
HER2-/BRMS1- (44)	78% DFS (104)

NOTE: Survival data were available for 212 cases. ER and PR staining data were available on 211 of these cases.

* DFS, disease-free survival.

[†] The lowest DFS for ER, PR, or HER2 is shown in boldface.

Table 4
HER2 overexpression in patient subgroups stratified by hormone receptor and BRMS1 status

Patient subgroup	HER2 positive	HER2 negative
ER ($P = 0.001$)		
ER+/BRMS1+	9 of 145 (6%)	136 of 145 (94%)
ER+/BRMS1-	7 of 42 (17%)	35 of 42 (83%)
ER-/BRMS1+	9 of 33 (27%)	24 of 33 (73%)
ER-/BRMS1-	5 of 17 (29%)	12 of 17 (71%)
PR ($P = 0.01$)		
PR+/BRMS1+	7 of 117 (6%)	110 of 117 (94%)
PR+/BRMS1-	4 of 27 (15%)	23 of 27 (85%)
PR-/BRMS1+	11 of 61 (18%)	50 of 61 (82%)
PR-/BRMS1-	8 of 32 (25%)	24 of 32 (75%)

Table 5

BRMS1 genomic copy number status and immunohistochemical staining

Genomic status	BRMS1 immunohistochemical staining intensity				Total
	0	1+	2+	3+	
BRMS1 deletion	2	0	0	0	2
BRMS1 euploidy	5	2	4	3	14
BRMS1 amplification	1	0	2	4	7
Total	8	2	6	7	23