Original Study

Association of Serum Progranulin Levels With Disease Progression, Therapy Response and Survival in Patients With Metastatic Breast Cancer

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Abstract

Progranulin (GP88) is a breast tumorigenesis driver. High tumor expression is associated with increased recurrence and mortality. Correlation between serum GP88 with survival and disease status was examined in 101 patients with metastatic breast cancer. Serum GP88 levels correlated with survival, therapy response, and disease progression. These data would suggest serum GP88 measurement to monitor disease status.in the standard of care.

Background: Progranulin (GP88) is a critical player in breast tumorigenesis. GP88 tumor expression is associated with increased recurrence and mortality, whereas GP88 circulating levels are elevated in patients with breast cancer compared with healthy individuals. We examined here the correlation between serum GP88 levels in patients with metastatic breast cancer (MBC) with overall survival and disease status determined as response to therapy or progression of disease. **Patients and Methods:** An institutional review board (IRB)-approved study prospectively enrolled 101 patients with MBC at the University of Maryland Marlene and Stewart Greenebaum Comprehensive Cancer Center. GP88 serum levels were correlated with patients' disease status determined by Response Evaluation Criteria In Solid Tumors (RECIST) 1.1 criteria and survival outcomes by Kaplan-Meier analysis and log rank statistics. **Results:** Patients' survival was stratified by serum GP88 level. Patients with serum GP88 $<$ 55 ng/mL had a 4-fold increased survival compared with patients with GP88 $>$ 55 ng/mL. Examination of GP88 serum levels in association with disease status showed a statistically significant association between serum GP88 levels and disease progression or response to therapy while CA15-3 level was only associated to progression. **Conclusion:** The association of serum GP88 level with survival and disease status suggests the potential of using the serum GP88 test for monitoring disease status in patients with MBC. Measurement of serum GP88 levels in patients with MBC may have clinical value as a cost-effective adjunct to the management of patients with MBC with imaging.

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Introduction

Despite a recent decline in breast cancer (BC) mortality, approximately 250,000 women are diagnosed annually with breast cancer in the United States alone.[1] Since the 1990s, consistent improvement in 5-year relative survivals and decrease in BC mortality have been observed, with a 1.9% average annual decline during the 2004 to 2013 periods owing to progress in therapy and improved detection and management of patients.[2,3] Of the ~250,000 cases of BC, only ~6% present with de novo stage 4, metastatic BC (MBC) at the initial diagnosis, whereas up to one-third have axillary lymph node involvement at time of diagnosis

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and are at substantially higher risk for progression to MBC.[4,5] Yet, of the ~40,600 annual deaths attributable to BC in the United States, a majority is owing to MBC, even though 92% of patients with BC present with localized or regional disease at the time of their primary diagnosis.[6] Five-year relative survivals for patients with local and regional disease at diagnosis are excellent: 99% and 85%, respectively, whereas only 27% of patients with MBC survive 5 years after diagnosis of advanced BC.[7,8] MBC is considered a mostly incurable disease and poses several challenges to the clinical management as a balance is sought between reducing treatment toxicity, providing improved quality of life, and improving overall survival.[9-11] Thus, the ability to detect and monitor metastatic disease is important in the overall management of BC.

The present approaches to monitor the efficacy of treatments for MBC during and post-therapy are multi-faceted but show limitations in enabling clinicians to successfully manage this disease. Clinical, laboratory, and imaging follow-up visits for monitoring patients with BC are the key components of ongoing disease management and are important not only to detect recurrence(s) and progression but also to monitor therapeutic response.[12] Computerized tomography (CT) scans and other imaging technologies allow evaluation of response to therapy or stability of disease while on therapy. Taken together with physical clinical examination and symptom evaluation, imaging assessment constitutes the mainstay of follow-up and monitoring of disease status in MBC.[13] Standardized response criteria such as Response Evaluation Criteria in Solid Tumors (RECIST 1.1) utilize serial imaging tumor measurements to determine response to anticancer therapy.[14] However, there are considerable variations regarding the nature, frequency, and type of follow-up imaging and testing needed for MBC. The National Comprehensive Cancer Network (NCCN) suggests that staging evaluation of women with recurrent BC or MBC should use diagnostic chest CT, bone scan, and radiographs of long bones.[15] CT of the abdomen with or without the pelvis may also be considered for restaging. Positron emission tomography/CT is considered an optional modality in situations where standard imaging results are equivocal or suspicious.[6,13,16] Although imaging represents the gold standard, it remains expensive, time consuming, and slow to detect disease response or progression.[17] Depending on the type of imaging, costs can range from $100 to $5,000, and overall, the costs to health care annually may be ~$150 to $200 million.[10,18] Enumeration and analysis of circulating tumor cells have been considered an additional strategy in the follow-up of patients in the metastatic setting as it can predict clinical outcome in conjunction with imaging.[19-22]

As an adjunct to imaging, cost-effective measurements of circulating tumor-associated biomarkers have been implemented to monitor MBC disease status.[23-25] Although serial monitoring of serum tumor biomarkers such as cancer antigen (CA) 15-3, CA27-29, and carcinoembryonic antigen (CEA) can be a useful adjunct to standard imaging methods for following response to therapy, the American Society of Clinical Oncology (ASCO) does not recommend their routine use for screening for recurrence or to implement anticancer therapy changes.[26,27] The current serum tumor markers provide clinicians with some measure of real-time disease progression (eg, CA15-3 is elevated in 70%-75% of MBC, whereas CEA is elevated in 40% of MBC).[28] In follow-up, CEA and CA15-3 have been shown to detect 40% to 60% of recurrences before clinical or radiologic evidence of disease with a lead-time between 2 and 18 months. Simultaneous use of both serum markers allows early diagnosis of metastases in up to 60% to 80% of patients with BC.[28,29] Combining the results of these 2 biomarkers improves the potential of this approach and helps identify progression of disease before imaging progression is noted.[30] However, even with recent reports and studies,[31,32] the clinical use of these biomarkers alone for identifying disease progression remains limited and is not recommended as an established criterion of response to anticancer treatments for MBC. Thus, there is need to identify additional circulating biomarkers that can complement those already measured in the standard of care. It is thought that the monitoring of real-time biological processes through measurement of biological markers that are drivers of the disease may provide a clearer understanding of the disease status and enable proactive clinical management. These disease "driver" biomarkers should improve real-time assessment of MBC disease status. Identifying such drivers of disease and developing their use as monitoring tools are therefore worthwhile strategies. Our laboratory has characterized a target biomarker, GP88, also known as progranulin (PGRN), granulin-epithelin precursor, acrogranin or PC-cell derived growth factor, which is expressed in BC tumor tissue and secreted in the circulation of patients with BC. Biological and clinical studies have established the importance of GP88/PGRN in BC tumorigenesis and as a predictive marker for recurrence. Published studies have demonstrated GP88 as a biological driver of tumor cell proliferation, survival, invasiveness, and drug resistance.[33-36] Both tissue and blood tests have been developed for determining GP88 expression in tumor tissue and measuring GP88 in biological fluids. Use of these tests has shown that: (1) GP88 is present in breast tumor tissue whereas it is not present in corresponding "normal" breast tissue[37]; (2) Increased expression of GP88 in estrogen receptor-positive breast adenocarcinoma cell lines is associated with estrogen independence and resistance to anti-estrogen therapy and aromatase inhibitors,[38,39] whereas in human epidermal growth factor receptor 2-overexpressing BC, GP88 stimulated human epidermal growth factor receptor 2 phosphorylation and conferred herceptin resistance[40]; (3) Increased tumor GP88 expression measured by immunohistochemistry in breast tumor tissue is an independent predictor of recurrence and is associated with poor outcome[41,42]; and (4) circulating levels of GP88 are measurable by enzyme immunoassay (EIA) and, compared with healthy individuals, elevated GP88 blood levels are found in patients with BC.[43]

Because high GP88 tissue expression is associated with poor outcome including increased risk of recurrence and mortality and because GP88 stimulates hallmarks of metastasis,[44] we have hypothesized that GP88 circulating levels could be used in patients with MBC to monitor disease status. This possibility was investigated in the present study that examined the correlation between GP88 serum levels and overall survival in patients with MBC.

Material and Methods

Patient Population

An institutional review board (IRB)-approved study at the University of Maryland Greenebaum Comprehensive Cancer Center (UMGCCC) prospectively enrolled 101 patients with stage 4 BC

(MBC) who signed informed consent forms for serial blood sampling while on standard therapy for BC. Study eligibility criteria included histologically confirmed diagnosis of BC, age ≥ 18 years, stage 1 to 4 at primary diagnosis, completed primary surgery, and radiation for treatment of BC. Patients were eligible to participate in this study irrespective of the number and types of prior therapies received for treatment of MBC. All patients received standard of care systemic therapy (endocrine, chemotherapy, immunological, or combined treatment) and follow-up. Patients were anonymized and assigned sequential study-specific numbers. Patient and tumor informations were collected from de-identified medical records. Blood sampling occurred during standard of care medical oncology visits at the time of routine blood draws required for standard BC follow-up. For patients on chemotherapy, blood samples were typically collected on treatment day before the start of chemotherapy, whereas patients on hormonal therapies were sampled during treatment. At time of each visit, non-fasting blood from patients was collected into serum preparation tubes (BD Diagnostics) at the UMGCCC and immediately transferred on ice, to A&G Pharmaceutical Inc in Columbia, MD for serum preparation, aliquoting, storage at −80°C and subsequent analysis of the serum GP88 levels. The attending medical oncologist determined disease status in line with RECIST 1.1 criteria as assessed by physical examination and imaging analysis. When a patient was clinically assessed without imaging being carried out, the disease status was reported based on the previous RECIST criteria determined for the patient.

Study Database

A password-protected clinical database of study participants was established at the UMGCCC following the University of Maryland IRB and Health Insurance Portability and Accountability Act (HIPAA) guidelines. Participants were de-identified and assigned unique study numbers. Patient demographics, clinical history, and medical findings, together with tumor and disease characteristics, were tabulated for further analysis. Serum GP88 and CA15-3 levels were entered in this database under the direction of the clinical principal investigator at the UMGCCC. The statistical analysis of the de-identified data was performed by an independent study statistician (DH).

Routine Serum CA15-3 Determination

UMGCCC routinely measures the blood level of CA15-3 on patients with stage 4 disease. CA15-3 was quantified as part of the patients' standard of care using the Ortho-Clinical Diagnostics CA 15-3 test kit run on the Vitros Clinical Analyzer at UMGCCC. Values of CA15-3 were added to the patient database described earlier.

Serum GP88/PGRN Enzyme Immunoassay

Whole blood samples received at A&G from UMGCCC were centrifuged at 1000 × g for 15 minutes at 4°C to collect serum. Aliquoted serum samples were kept at −80°C before assaying for GP88. Measurement of serum GP88/PGRN levels was carried out in triplicate using a sandwich EIA developed in our laboratory using a combination of capture and detecting anti-human GP88 antibodies with increasing amounts of purified human recombinant GP88 used as standard for calibration curve.[43] Serum calibrators consisting of human sera alone or spiked with known amount of GP88 were also used as internal controls in the assays. Specifically, 96-well EIA plates were coated with 1 μg/well of capture anti-GP88 mouse monoclonal antibody at 4°C overnight and subsequently blocked with non-fat milk. After washing, serum samples or standard human GP88 (0-20 ng/mL) were added and incubated for 2 hours at 37°C. Following washing, 100 μL of detecting rabbit anti-human GP88 antibody (10 μg/mL) was added and incubated at 37°C for 1 hour. Following washing, horseradish peroxidase-conjugated goat anti-rabbit secondary IgG was added. After 1 hour incubation, plates were washed, and 100 μL of horseradish peroxidase substrate was added. OD at A_{620} was read with a microtiter plate reader. Amount of GP88 in serum samples was calculated from the standard human GP88 curve.

Statistical Analysis

Descriptive statistics were used to summarize patients' characteristics. These were reported by proportions (for categorical variables) and median (range) for continuous variables. For the determination of the threshold serum GP88 values associated with changes in survival outcomes, all serum GP88 values were used, and survival outcomes was determined using the survival time from GP88 measurements to last follow-up or death. R, a language and environment for statistical computing and graphics, was used for the statistical calculations. R release 3.3.0 was used for calculations in this study. The R package survival was used for Kaplan-Meier analysis, to fit Cox proportional hazards (CPH) models and compute log rank test statistics.

Results

MBC Patient Populations

The 101 patients with MBC enrolled in the study at UMGCCC Breast Clinic were treated and followed longitudinally per standard of care by physical examination, laboratory assays, and imaging, with a total of 262 clinical data points over the period of study with an average of 2.6 images per patient (range, 1-11). This information was used by the attending clinician to provide a disease status as defined by RECIST 1.1. As described in the method section, when blood samples were obtained without a simultaneous imaging, the RECIST assessment from the previous visit was used.

Table 1 provides descriptive statistics of the patients enrolled in the study for age, race, and tumor and disease characteristics at initial diagnosis, including hormone receptor expression, tumor size, tumor grade, and lymph node status, as well as number and types of metastasis sites at study entry.

The patient and tumor information indicated that the enrolled patient population was representative of a general population of patients with MBC. The median follow-up for stage 4 patients in this study was 38 months (range, 1-200 months). The median survival was 17.8 months. Twenty-nine percent of the patients had single-site metastasis (52% of single site being bone metastasis and 26% being lung metastasis), whereas 71% of patients with MBC had multiple metastasis sites, with bone and lung metastasis being the most common metastatic sites.

It should be noted that the African American patient population (48%) was well represented in this study, considering that African Americans represent 30% of the state of Maryland population.

Table 1 Patient Demographics and Tumor Characteristics

Age, y		**Race**	n (%)			
Mean	54	Caucasian	51 (50)			
Median	54	African-American	48 (48)			
Range	29-86	Other	2 (2)			
Tumor size at initial diagnosis[a]	n (%)	**Hormone receptor/ HER2 status**[a]				
T1	19 (19)		Caucasian, n (%)	African-American, n (%)	Other, n (%)	Total, n (%)
T2	15 (14)	ER^+	31 (61)	27 (56)	2 (100)	60 (59)
T3	19 (19)	PR^+	19 (37)	25 (52)	0 (0)	44 (44)
T4	0 (0)	ER^+PR^+	18 (35)	24 (50)	0 (0)	42 (42)
Unknown	48 (48)	ER^-PR^-	19 (37)	24 (50)	0 (0)	43 (43)
		ER or PR unknown	3 (3%)	0 (0)	0 (0)	3 (3)
Axillary lymph node at initial diagnosis[a]	n (%)	$HER2^+$	30 (59%)	35 (73)	2 (100)	67 (66)
Positive	40 (40)	$HER2^-$	15 (29%)	11 (23%)	0 (0%)	26 (26)
Negative	21 (20)	HER2 unknown	6 (12)	2 (4)	0 (0)	8 (8)
Unknown	40 (40)	Triple negative	6 (6)	13 (13)	0 (0)	19 (19)
Metastatic sites	n (%)		n (%)			
Visceral	14 (14)	≤2 sites	59 (58)			
Non-visceral	29 (30)	>2 sites	42 (42)			
Both	57 (56)					

Abbreviations: ER = estrogen receptor; HER2 = human epidermal growth factor receptor; PR = progesterone receptor.
[a]Tumor size and axillary lymph node status of patients were determined at initial diagnosis of breast cancer. All other parameters were determined at study entry. Visceral metastatic sites correspond to lung, liver, and organs of digestive, excretory, reproductive, and circulatory systems.

Determination of a Serum GP88 Threshold Level That Stratifies Patients for Survival Outcome

Blood collections were performed on patients at the time of routine visits, and these samples were assayed to determine serum GP88 level determination by EIA as described above. During the duration of the longitudinal study, 446 GP88 data points were obtained with an average of 4.3 (range, 1-19) blood GP88 determinations per patient. Taking each GP88 result, we calculated the time to death or last follow-up and then distributed the serum GP88 data by quartile (Q1 to Q4) as shown in the legend of Figure 1A. Using these data, Kaplan-Meier survival graphs for these GP88 level groups were plotted (Figure 1A). This graph indicated that the Q4 group of GP88 results corresponded to significantly shorter survival outcome compared with Q1 to Q3, all of which had similar survival outcomes.

As a second analysis of the serum GP88 values, Kaplan-Meier plots were performed using GP88 results grouped by 10 ng/mL increments (ie, 10-20, 20-30, 30-40 ng/mL etc). This analysis resulted in Kaplan-Meier plots to establish a serum GP88 value above which there was a sharp decrease in survival (Figure 1B). A further refinement of the threshold value for serum GP88 levels showed that a serum GP88 level of 55 ng/mL represented a threshold stratifying 2 groups with distinct survival outcomes.

Using the value of 55 ng/mL, of the 101 patients enrolled in the study, 91 patients who had 3 or more serial GP88 determinations were examined as described in the method section and stratified into 2 GP88 groups: 1 for whom the GP88 serum level remained above 55 ng/mL and 1 for whom the serum GP88 remained below 55 ng/mL. Kaplan-Meier survival analysis of the 2 patient groups (Figure 2) and log rank statistics investigated the statistical significance between the groups and indicated that there was a significant difference ($P = .03$) in survival between the high and low GP88 groups. The median survival of the low GP88 group was 20.7 months, whereas the median survival of the high GP88 group was 4-fold shorter at 4.8 months. The CPH model was used to examine the hazard ratio for both groups (Table 2). The estimated hazard ratio of 0.537 for the low GP88 group compared with the high GP88 group was statistically significant ($P = .027$) and clinically meaningful.

Thus, the 55 ng/mL serum level of GP88 can be selected as a cutoff level for stratification of patients for survival outcomes.

Stratification of Patients by Serum GP88 Level for Survival is Independent of Patient Demographics, Tumor, and Disease Characteristics

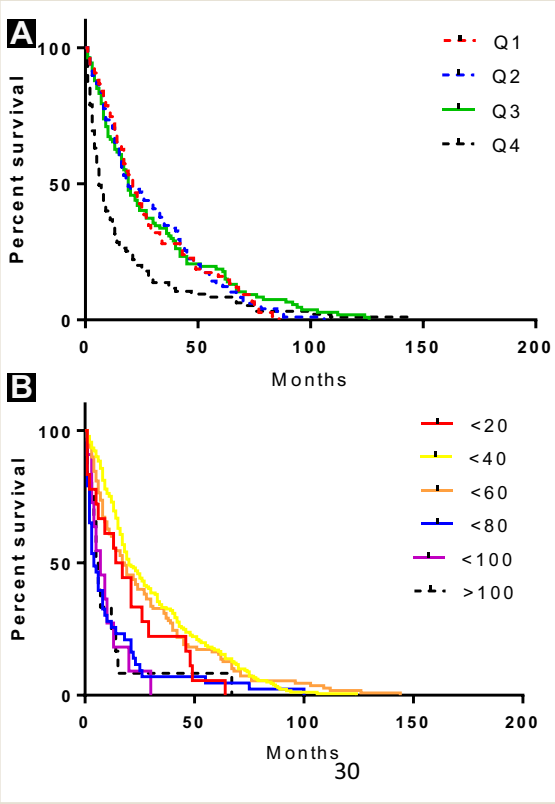
As shown in Table 1, enrolled patients presented diverse initial tumor and disease characteristics as well as demographics. Because 48% of the enrolled MBC population was African American, we also examined whether serum GP88 association with survival was observed in the African American population as well as in the Caucasian population.

Several tumor and disease risk indicators were measured to assess whether they were associated with survival, and whether GP88 provided significant additional information. This was tested by fitting CPH survival models using each predictor followed by the GP88 grouping.

The data in Table 3 provide the results of the sequential analysis of deviance.

Of the traditional tumor and disease predictors, only the number of metastases ($P = .0055$) and tumor grade ($P = .0314$) attained

Figure 1 Determination of a GP88 Threshold Value Associated With Changes in Survival Outcomes. A, Top Graph: Kaplan-Meier Survival Graph Determined by Serum GP88 Quartile. Serum GP88 Values Were Classified by Quartiles Calculated as the Following: Q1 < 44 ng/mL; Q2: 44-54 ng/mL; Q3: 54-60 ng/mL; Q4: >60 ng/mL. The Serum GP88 Q4 Group Was Associated With Significantly Shorter Survival Outcome Compared With Q1 to 3 Which Had Similar Survival Outcomes. B, Bottom Graph: Survival Outcomes for Serum GP88 Values Classified by 20 ng/mL Increments. Serum GP88 Levels < 60 ng/mL Showed an Improved Survival Compared With GP88 Values Above This Level



Abbreviations: GP88 = progranulin; Q = quartile.

statistical significance. The tests for additional information in GP88 were uniformly significant or close to significant in the CPH model that could avail themselves of all 101 cases (P values ranging from .0028 to .0599).

These calculations provide confirmation that GP88 provides significant additional survival information.

Association of Serum GP88 and CA15-3 Levels With Response or Progression of Disease

CA15-3, which detects soluble forms of MUC-1 protein, is a circulating tumor marker most widely used in the standard of care as a monitoring marker for disease progression for patients with stage 4 BC. In this analysis, serum GP88 and CA15-3 values were tested for association with contemporaneous assessment of disease response or disease progression determined by the attending clinician using RECIST 1.1 criteria. This association was examined by the Wilcoxon test. The results of these analyses are shown numerically in Table 4.

As expected, serum CA15-3 level was significantly associated with progression ($P < .0001$) but was not significantly associated with treatment response ($P = .7316$). Interestingly, GP88 was highly statistically associated with both response ($P = .0194$) and progression ($P = .0101$)

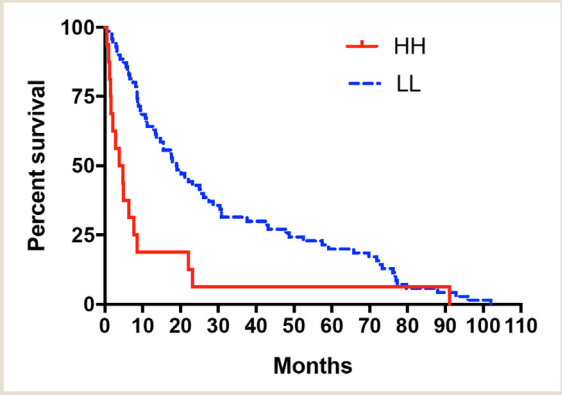
Further analysis investigated the additional information provided by one biomarker to the other in the association with disease progression or response using RECIST 1.1. The analysis was made using logistic regression. In this, progression or response was modeled as dependent on the log-transformed GP88 and CA15-3 values. The results of this analysis are shown in Table 5.

The logistic regression shows significance for both GP88 ($P = .0442$) and CA15-3 ($P = .0001$) for association with progression. This means that each of these biomarkers provides statistically significant additive information on progression. In contrast, only GP88 ($P = .0087$) shows high statistical significance for response. This means that GP88 alone is sufficient for monitoring treatment response and CA15-3 ($P = .6757$) does not add any value.

Discussion

Glycoprotein GP88 (progranulin) is a growth and survival factor playing an important role in BC tumorigenesis with high GP88 expression associated with increased tumorigenicity, drug resistance, and metastasis.[36] Biological, pathologic, or clinical studies from multiple laboratories have also established progranulin as an important player in multiple types of cancers.[45] We have previously shown that GP88 can be scored in tumor tissue by immunohistochemistry staining with the anti-human GP88 monoclonal antibody 6B3 developed in our laboratory. The data showed that GP88 was expressed at higher level in BC tissues (invasive ductal carcinoma), whereas it was negative in normal mammary tissue.[37] Pathologic studies with 600 estrogen receptor-positive invasive ductal carcinoma cases established that high GP88 tumor score (3+ by immunohistochemistry) was associated with a 4-fold increase in recurrence and a 2.5-fold decrease in overall survival, making GP88 an independent risk of recurrence predictor and a marker for poor outcome.[42] Because GP88 is a secreted protein, EIA to measure GP88 in biological fluids demonstrated that GP88 was measurable in serum and was found at a higher level in serum of patients with BC when compared with healthy individuals.[43] Increased tumor tissue GP88 and serum levels have been found to be associated with poor outcomes in non–small-cell lung carcinoma,[46] and serum GP88 was associated with Gleason score and poor outcome in patients with advanced prostate cancer.[47] Interestingly, increase of GP88 level in cerebrospinal fluid was reported in patients with cancer with central nervous system (CNS) metastasis compared with patients without CNS metastasis,[48] suggesting that this could be used as an approach to detect CNS metastases, which are sometimes difficult to determine by imaging. The present paper was focused in examining the association of serum GP88 level in stage 4 patients with BC with survival and whether measuring serum GP88 level could be used to monitor patient response to therapy or progression

Figure 2 Survival for Patients Stratified by the Serum GP88 Cutoff of ≤ 55 and > 55 ng/mL. Kaplan-Meier Analysis of Survival of Patients With Metastatic Breast Cancer Who Were Stratified Into 2 Groups Based on Their Serum GP88 Values. Hatched Blue Line (LL) Corresponds to Patients (74 Patients) Whose Serum GP88 Remained Below or Equal to 55 ng/mL. Solid Red Line (HH) Corresponds to Patients (17 Patients) With Serum GP88 Levels Remaining High > 55 ng/mL



Abbreviations: GP88 = progranulin; HH = serum GP88 levels > 55 ng/mL; LL = serum GP88 levels ≤ 55 ng/mL.

of disease. For this purpose, we carried out an IRB-approved prospective blood sampling study enrolling 101 patients with MBC for whom serum GP88 level was measured longitudinally along with determination of disease status using RECIST 1.1 criteria.

The results established a serum GP88 threshold level of 55 ng/mL that stratified patients for good or poor outcome independently of patients' age, race, and tumor/disease characteristics such as tumor size, estrogen receptor/progesterone receptor/human epidermal growth factor receptor 2 expression, and lymph node status. The number of metastatic sites had a highly significant impact on survival. However, GP88 provided significant additional information. Because serum GP88 level and metastasis numbers are both good risk predictors, these findings would suggest that GP88 and metastasis status provide separate predictive information on survival, which would be very useful for the management of patients. Patients with a serum GP88 level of ≤ 55 ng/mL had a 4-fold improved overall survival when compared with patients with serum GP88 level > 55 ng/mL. The reduction of hazard ratio in the low GP88 group (LL) when compared with the high GP88 group (HH) was significant. These findings would support the utility of serum GP88 measurement across diverse populations of patients with BC to contribute to the risk management of patients. The changes in serum levels of CA15-3, a circulating tumor marker used in the standard of care monitoring, have been mainly associated with disease progression rather than response to therapy. Even though the utility of measuring CA15-3 levels for patients with BC remains a subject of discussion, the European Group on Tumor Markers has recommended the use of CEA and CA15-3 levels for assessing prognosis, the early detection of disease progression, and treatment

Table 2 Cox Proportional Hazard Model for the Serum GP88 Groups

Term	Coefficient	Hazard Ratio	SE	z	*P*
GP88 LL group	−0.622	0.537	0.282	−2.21	.027

Cox proportional hazard compares the always-low serum GP88 LL group against the always-high serum GP88 HH group.
Abbreviation: GP88 = progranulin; HH = serum GP88 levels > 55 ng/mL; LL = serum GP88 levels ≤ 55 ng/mL.

Table 3 Relevance of Demographic and Disease Covariates to Survival

Cases	Events	Terms	Deviance	DF	*P*
101	96	ER	1.061	1	.3029
		Then GP88_grp	7.409	3	.0599
101	96	PR	0.251	1	.6162
		Then GP88_grp	7.945	3	.0472
101	96	HER2	1.177	1	.2779
		Then GP88_grp	7.689	3	.0529
101	96	Age	0.008	1	.9284
		Then GP88_grp	8.332	3	.0396
101	96	Race	5.777	3	.1230
		Then GP88_grp	14.042	3	.0028
101	96	Metastases	7.693	1	.0055
		Then GP88_grp	10.801	3	.0129
72	67	LNPos	2.964	1	.0851
		Then GP88_grp	6.670	3	.0832
69	66	Tumor size	2.076	1	.1496
		Then GP88_grp	3.755	3	.2892
69	68	Tumor grade	4.632	1	.0314
		Then GP88_grp	3.476	3	.3238

Covariates were examined to assess their association with survival, and whether GP88 had significant additional information. This was tested by fitting CPH survival models using each predictor followed by the GP88 grouping. The results are provided as the sequential analysis of deviance. For each predictor, the table shows the deviance explained by that predictor and the additional deviance explained by the GP88 group, along with the DF and *P* value of each. The first 2 columns show the number of patients and events recorded for each CPH fit. Information for the last 3 predictors were missing for a substantial fraction of the patients, which would explain the paucity of significances for these 3 later fits.
Abbreviations: CPH model = Cox proportional hazard model; DF = degrees of freedom; ER = estrogen receptor; GP88 = progranulin; HER2 = human epidermal growth factor receptor 2; LNPos = lymph node positive; PR = progesterone receptor.

Table 4 Biomarker Performance in Identification of Progression or Response to Therapy Relative to Contemporaneous RECIST Criteria

Predictor	Dependent	*P* Value
GP88	Progression	.0101
GP88	Response	.0194
CA15-3	Progression	<.0001
CA15-3	Response	.7316

The 2 biomarkers were tested for association with contemporaneous RECIST assessment of disease progression and response to therapy. The values of these predictors were tested for statistical significance of the difference between the 2 groups using the Wilcoxon test. Table 4 shows that the contemporaneous GP88 is highly significantly associated with disease progression and response, whereas CA15-3 is only significant in conjunction with progression.
Abbreviations: GP88 = progranulin; RECIST = Response Evaluation Criteria In Solid Tumors.

monitoring in BC. In contrast, the ASCO and the NCCN guidelines do not currently recommend routine use of serum CA15-3 and CEA for BC screening and directing treatment, although recent papers and studies are reevaluating the clinical applicability of CEA and CA15-3.[26,27] This emphasizes the continued interest in identifying novel circulating biomarkers such as GP88 that can be used as cost-effective adjuncts to imaging for assessing disease status and response to therapy in patients with MBC.[25] In this context, we examined the association of serum GP88 levels with either response to therapy or progression of disease and compared the results with the ones obtained for CA15-3. We show here that serum CA15-3 was strongly associated with progression of disease but not with response to therapy. Interestingly, serum GP88 measurements were statistically associated not only with progression of disease but, more importantly, with response to therapy. Moreover, the information provided by GP88 on disease status (progression or response to therapy) was additive to the one provided by CA15-3 in this study population. It is interesting to note that the application of serum GP88 determination is not limited to patients with stage 4 BC because Koo et al had demonstrated that serum progranulin levels were clinically significant for predicting recurrence in patients with hormone-positive BC during adjuvant therapy.[49] The fact that tissue GP88 level is also predictive of recurrence in patients with hormone-responsive BC would suggest the possibility of complementarity between tissue and serum progranulin tests for the management of patients with early stage BC. The present study would also suggest that serum GP88 determination has the potential to have additional clinical utility in the standard of care alongside CA15-3 to provide important information for patient management that other current serum markers cannot provide.

Table 5 Complementarity of GP88 and CA15-3 Performance in Identifying Disease Progression or Response

Dependent	Biomarker	*P* Value
Progression	GP88	.0442
Progression	CA15-3	<.0001
Response	GP88	.0087
Response	CA15-3	.6757

Using logistic regression techniques, progression or response was modeled as dependent on the log-transformed GP88 and CA15-3 values. This enabled us to examine the additional information provided by one biomarker to the other in the association with disease progression or response using RECIST 1.1. The logistic regression shows significance for both GP88 (P = .0442) and CA15-3 (P = .0001) for association with progression. This means that each biomarker provides statistically significant additive information on progression. When examined for association with response, only GP88 (P = .0087) showed high statistical significance. This means that GP88 alone is sufficient for monitoring treatment response and CA15-3 (P = .6757) does not add any value.
Abbreviations: GP88 = progranulin; RECIST = Response Evaluation Criteria In Solid Tumors.

Conclusion

At present, determination of response to therapy during treatment for MBC is carried out by using various imaging techniques repetitiously, which are typically performed at intervals between 6 and 12 weeks, causing increased exposure of patients to radiation and significantly increasing the overall cost. The ability to have multiple serum biomarkers to complement these imaging assessments by providing more frequent, less invasive, and more cost-effective measurements is clinically attractive and warrants further investigation. A prospective study in patients with MBC is planned to explore these possibilities.

Clinical Practice Points

- The ability to monitor disease status (ie, response to therapy or progression of disease) in patients with metastatic cancer in the standard of care is of high importance to oncologists to evaluate whether a treatment is adequate or need to be changed. For this purpose, the availability of minimally invasive and cost-effective tests to monitor disease status is attractive to medical oncologists and to patients. With tests such as CA15-3 or CEA at their disposal, oncologists are accustomed to using the assessment of tumor biomarkers levels alongside imaging and physical examination for the management of patients.
- Here, we show that the serum levels of GP88 (progranulin) which is a marker of disease aggressiveness, are associated with survival, progression of disease, and response to therapy.
- Considering that CA15-3 assays are only associated with progression of disease, measurement of GP88 has the potential to provide additional data to the ones provided by the CA15-3 tests used in the standard of care should be on interest to medical practitioners in this field of oncology.

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Disclosure

G. Serrero, D. Hicks, and B. Yue performed this study while employees of A&G Pharmaceutical. G. Serrero is also a shareholder of A&G Pharmaceutical. The remaining authors have stated that they have no conflicts of interest.

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