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Short Communication

Is There a Correlation between Androgen Receptor (Ar) Expression in Er+/Her2- Metastatic Breast Cancer and Response to Anti-Estrogen Treatment? A Pilot Study

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Abstract

Background: There is recent interest in the use of antiandrogen therapy to treat metastatic androgen receptor positive (AR+) 'triple negative' metastatic breast cancer. Since AR testing is not routinely done, we conducted a pilot study to determine whether there was any evidence of a correlation between AR expression and response to antiestrogen treatment in estrogen receptor positive (ER+) metastatic breast cancer (MBC) patients. Such a correlation might identify ER+ MBC patients who are candidates for AR testing and a trial of antiandrogen therapy for AR+ tumors.

Methods: A retrospective study was conducted of 46 randomly selected patients treated with anti-estrogen therapy for ER+ Her2-ve MBC who had an available tumor biopsy. Immunohistochemical assessment of AR expression was performed on all available primary and metastatic tumor specimens for each patient. Patients were classified into two categories according to their total duration of clinical benefit from anti-estrogen treatment: A) ≥ 6 months (m) and B) <6 m.

Results: AR was +ve in all 20 primary tumor samples available and in 85% (40/47) of the metastatic lesions. There were 39 (85%) and 7 (15%) patients in endocrine response categories A and B respectively. No statistically significant differences were found between the presence and intensity of AR staining in the metastases and the duration of benefit from hormone therapy.

Conclusion: Our results do not suggest that response to anti-estrogen treatment can determine possible candidates for AR testing and a trial of anti-androgen therapy for ER+ AR+ HER2- patients. A significant minority of metastases from ER+ AR+ primary tumours become AR-.

INTRODUCTION

Approximately 75-80% of metastatic breast cancer (MBC) patients are estrogen receptor (ER) positive, and/or progesterone receptor (PR) positive and over the last few decades this subgroup has been representing an increasing proportion of all breast cancer cases [1]. The ER signaling pathway has been extensively studied, and targeting this pathway with antiestrogen agents has been a cornerstone of the treatment of these patients for several decades. Although the majority of patients with ER/PR+ MBC will respond to endocrine therapy, approximately 25-30% of these patients will not obtain clinical benefit. Even among responders, median duration of response with currently available agents is less than one year, [2] and shorter durations of progression-free survival are seen with each successive line of endocrine therapy. Median survival of ER+ MBC patients is only two to three years [3]. Thus it is essential to find active well-tolerated treatments to improve the survival and quality of life of these patients.

Although androgen receptor (AR) positivity in breast

carcinomas was described decades ago [4] and pharmacological doses of androgens were used successfully in the past for the treatment of metastatic ER+ breast cancer [5,6], relatively little was known about the mechanism of action of such treatment. However, in recent years new clinical and molecular findings have renewed the interest in this pathway and its potential as a therapeutic target. Overall, the expression of AR in breast cancer is common and ranges from 60 to 89% in various series [6-14]. However, striking differences in AR expression are found when cancers are classified according to molecular phenotype. AR expression is most common in Luminal A and B tumors (68% to 95%), followed by Her2 positive tumors (40% to 56%), while triple negative tumors have a much lower rate of expression ranging from 10% to 32%, with many of these tumours having apocrine features [5,9,10,15].

Despite the remarkable high prevalence of AR expression, its clinical significance and role are still unclear. The AR is a member of the steroid hormone receptor family that, once bound by a ligand, forms a homodimer that translocates to the nucleus and promotes a cascade of molecular events resulting in the activation of target gene transcription [16]. Androgens have shown an effect on breast epithelial cells indirectly through the activation of estrogen-responsive genes after conversion to estrogen by aromatase, or directly via the AR, independent of estrogen and progesterone receptors, which may explain the different responses to androgens found in preclinical models.

In the past few years, the AR has become a new area of research for targeted therapies. A group of investigators at Sloan-Kettering Memorial reported a 21% response rate to bicalutamide 150 mg daily in a phase 2 study of 26 metastatic 'triple negative' breast cancers expressing the AR (AR+) [17]. Ongoing trials are testing next generation compounds such as Enzalutamide or Abiraterone for AR+ tumors. Recently, our group reported the case of a patient with 'triple negative' AR+ MBC who, having progressed following several lines of chemotherapy, achieved a complete and prolonged response to Bicalutamide 150 mg daily [18].

AR blockers might have clinical benefit, not only in the small subset of AR+ 'triple negative' tumours, but in the much larger group of ER+ AR+ tumours. Patients with ER+ metastatic disease often have prolonged responses to sequential hormonal therapies but ultimately `run out` of options and must proceed to chemotherapy, which is more toxic and less convenient. It would be very beneficial if such patients could receive an additional line of effective endocrine therapy either to delay the need for chemotherapy or to enable them to have a `chemotherapy holiday` without the fear of tumor progression in the absence of treatment. Antiandrogen therapy might also be an option for the sizable proportion of ER+ patients with metastatic disease who do not respond to antiestrogen therapy.

The primary goal of this pilot study was to correlate the rate and intensity of AR expression with response to antiestrogen therapy in a sample of patients with ER+ HER2 –ve MBC treated at our center. Since AR testing is not routinely done on breast cancers, such a correlation might identify ER+ MBC patients who are candidates for AR testing and a trial of antiandrogen therapy for tumors found to be AR+. Secondary aims were to correlate the expression of AR in the primary tumors and their metastases as well as between metastases taken from the same patient. This information could help determine whether biopsy of a metastatic lesion is necessary when a trial of antiandrogen therapy is being considered.

MATERIALS AND METHODS

A convenience sample was randomly selected of female ER+ Her2-ve MBC patients treated at our center with anti-estrogen therapy for a minimum of 3 months, between January 2008 and December 2011. Patients could be primary MBC cases or metastatic relapses from initially early-stage tumors. Tumor tissue from either the primary tumor and/or from at least one metastatic site was also required.

Clinical benefit from anti-estrogen treatment was measured as progression free survival (PFS) based on clinical, imaging and biochemical parameters. The patients were classified in two categories according to their total duration of clinical benefit (tumour shrinkage or stabilization) from anti-estrogen treatment:

A. Good response: greater than 6 months (m)

B. Poor response: less than 6 months

Patients whose response to treatment was not adequately reported or who were lost to follow-up were excluded from the analysis.

Immunohistochemistry to assess AR expression was performed at our centre on all available tumor specimens for the selected patients using AR Leica antibody SP107, 1:50 dilution. To expose target proteins antigen retrieval was performed for 64 minutes in CC1 buffer (pH 8). Antibody incubation time was 36 minutes on the Ventana benchmark instrument. Nuclear staining for AR was detected by the DAB Ultra View detection system on one representative block per case. The percentage of cells staining and the intensity of AR expression were evaluated subjectively against a positive control of prostate tissue, in a manner similar to that used for evaluation of estrogen and progesterone receptor expression. Intensity was defined as AR –ve if there was <1% expression, and then 'weak', 'moderate' or 'strongly' positive for AR expression, the latter classification used if staining intensity was similar to the positive control. [19,20] For the analysis the 'weak' and 'moderate' cases were combined and compared to the 'strong' cases.

A convenience sample of approximately 50 patients was planned for this study. For descriptive purposes, continuous variables have been summarized as mean, median and standard deviation (error). Categorical variables are presented as proportions with a 95% confidence interval. The correlations between AR expression and endocrine response were measured by Fisher's exact test.

RESULTS

A total of 46 female patients met all the inclusion criteria. The median age was 58 years (range 31 to 90 years). Invasive carcinoma of no special type (NST) was the most common histological type, and was present in 31 (67%) of the cases.

The rest were invasive lobular carcinoma. The majority of the specimens (79%) were intermediate grade, 17% were high grade and 4% were low grade

The majority of the patients (85%) had a good response to anti-estrogen therapy, and 15% had a poor response. Patient demographics and tumor characteristics are summarized in Table 1.

A total of 67 samples from 46 patients were analyzed (Figure 1). Of the 67 samples, 20 corresponded to the primary tumor from 20 patients, while the other 47 samples were from a metastatic site from 36 different patients. Ten patients had samples from both primary and at least one site of metastasis (Table 2). The sites of metastasis were diverse and included: skin, bone, pleura, lung, liver, lymph nodes, peritoneum, stomach, soft tissue or bladder. The most common sites of metastasis were skin and bone.

AR was +ve in 100% of the 20 primary tumor samples and 85 % (40/47) of the metastases. Seven samples from metastatic sites from seven different patients were found to be AR –ve, six from patients who had a good response to hormone treatment and one from a patient with a poor response to treatment. Three of the negative samples were cytologic specimens from a pleural effusion and one from a bone metastasis. The other three samples were from endometrium, skin and liver.

AR expression in the primary and metastatic site was concordant in 70% (7/10) of the samples. In three cases the metastatic samples from pleural fluid (two cases) and endometrium were AR -ve while the primary was AR+. One of the patients had discordance between two different metastatic sites (endometrium AR-ve and skin AR+) (Table 2). Eight patients in total had more than one sample from a metastatic site. A patient for whom no primary tumor tissue was available had AR+ disease in bone but AR-ve disease in skin. Concordance for AR expression was found in the metastases of the other 6 patients with samples from more than one metastatic site

Intensity of AR expression was measured in all 60 AR+ samples and was found to be 'strong' in 53 (88%) of the samples, 'moderate' in 5 (8%) and weak in 2 (3%).

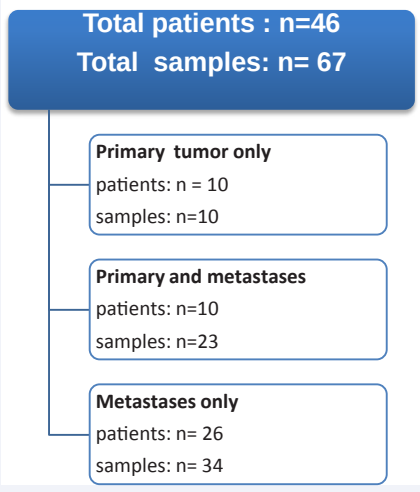

Figure 1 Flow chart of patients and samples analyzed.

Table 1: Patient and tumor characteristics.

Median age (range) (n=46)	**58 (31-90)**
Response to endocrine treatment • > 6 months. • < 6 months.	 85% (39/46) 15% (7/46)
Samples (n=67) • Primary • Metastases	 20 47
Histological subtype • IC NST • ILC	 66% (37/56) 34% (19/56)
Tumour Grade • Grade I • Grade II • Grade III	 4% (2/53) 79% (42/53) 17% (9/53)

Abbreviations: IC NST: Invasive Carcinoma No Special Type; ILC: Invasive Lobular Carcinoma

The correlation between anti-estrogen therapy response and AR staining among the patients for whom there was at least one metastatic specimen was investigated (Table 3). The two patients for whom there was discordance between the two sites of metastases were considered to have AR-ve disease. AR positivity was found in 82% of the cases with a good response to therapy, and in 91% of those with a bad response No statistically significant differences were found between these two groups of responders (good *vs.* poor) for either presence/absence of AR expression (p=0.459) or intensity (strong vs. moderate/weak) of the staining (p=0.257).

DISCUSSION

In this pilot study no significant association was found between AR expression and response to anti-estrogen treatment in ER+ MBC patients. In 1979 Allegra et al. [21] reported the findings from a retrospective study examining the association between AR expression and responsiveness to anti-estrogen therapy for 54 patients with metastatic or localized inoperable breast cancer. Of the 19 patient with AR+ tumors, 11 (58%) responded to treatment, while 11 of 35 (31%) with AR-ve disease responded (p=0.11). In another retrospective study, Bryan et al. [22] found a statistically significant association between AR expression and improved response to tamoxifen with 54% of AR+ patients responding compared to 14% of AR- patients (p <0.05). However, in those studies AR expression was not determined by immunohistochemical staining, but by cytoplasmic protein quantification. A tumor was considered to be AR+ when over 10 fmol of 3H-dihidrotestosterone per mg of cytoplasmatic protein was found. Moreover, today the great majority of patients with metastatic disease are treated first line with aromatases inhibitors. This makes results of these older studies difficult to compare to those of more recent studies such as ours. One strength of our study is the correlation between response to hormone treatment and AR expression, using current hormonal therapies and state-of –the art methods of AR evaluation. To the best of our knowledge no recent studies other than ours have

Table 2: Concordance of AR expression and staining of primary and metastatic samples.

PT	RESPONSE (m)	Site	AR(+/-)	AR%	AR intensity
1	>6	Breast	+	100	strong
		Omentum	+	100	strong
		Gastric	+	100	strong
2	>6	Breast	+	100	strong
		Pleural effusion	Negative	0	absent
3	>6	Breast	+	100	strong
		Skin	+	100	strong
4	>6	Breast	+	100	strong
		Endometrium	Negative	0	absent
		Skin	+	100	strong
5	>6	Breast	+	100	strong
		Bone	+	90	strong
6	>6	Breast	+	100	strong
		Bone	+	100	strong
7	>6	Breast	+	100	strong
		Bone	+	90	strong
8	<6	Breast	+	100	strong
		Pleural effusion	+	10	weak
9	<6	Breast	+	100	strong
		Pleural effusion	Negative	0	absent
10	<6	Breast	+	50	strong
		Skin	+	100	strong

Abbreviations: AR: Androgen Receptor; M: Months;

Table 3: AR staining intensity of metastases according to response to total duration of antiestrogen treatment (n=36).

Antiestrogen Response	AR staining intensity			
	Strong	Moderate	Weak	Absent
≥ 6 m (n=30)	19 (63%)	4 (13%)	1 (3%)	6 (20%)
<6m (n= 6)	3 (50%)	1 (17%)	1 (17%)	1 (17%)

assessed the role of AR in predicting response to endocrine therapy in the metastatic setting.

Our study found a high prevalence and intensity of AR expression in both the primary and metastatic tumors of patients with ER+ HER2 –ve MBC. None of the primary tumors and only 15% of the samples from metastatic sites was found to be AR –ve. Three of the AR -ve samples was cytologic specimens from pleural effusions and one was a bone biopsy. Therefore, in four of the seven negative cases the processing procedure could have influenced these results. Bone samples undergo a decalcification process that may hamper immunohistologic investigation, ruining the antigenicity of the tissue [23]. The accuracy of AR immunohistochemistry on cytological specimens from pleural effusions is uncertain. We found no previous studies reporting the prevalence of AR+ expression in malignant pleural effusions from MBC patients, but it is well known that the use of immunohistochemistry on cytologic samples offers several limitations compared to biopsy samples and could lead to false negative results. Similarly, in two of the three cases in which a metastatic site was AR- while the primary tumor was AR+, the negative site was a pleural effusion. However, the finding of loss of AR in biopsies from metastases to endometrium, skin and liver, as well as in a metastatic site from two patients with an AR+ metastasis in a different site, suggests that loss of AR expression, like loss of ER+ expression, does occur in the metastases of a significant minority of patients. Since the metastases of all patients in our series were ER+, we do not know whether the AR tends to be lost when an ER+ primary tumor produces an ER -ve metastasis.

Recently Cimino-Mathews et al. [24] reported their findings in two cohorts of patients in whom AR expression in metastatic lesions was retrospectively examined and compared to the primary tumor. The first cohort consisted of 16 patients who had surgically resected metastases. Twelve of the 16 primary tumors were AR+ and this expression was maintained in 92% (11/12) of the matching metastases. In only one of the four

cases of AR-ve primary tumors (which were all 'triple negative') was a metastasis AR+. The second cohort consisted of 16 patients from whom metastases were harvested at autopsy and matched to their primary tumor. In this series 11 (69%) of the primary tumors were AR+ but AR positivity was only observed in 7 (45%) of the metastases. These findings suggest that as the disease progresses, changes in the AR expression of the tumor may take place, which could be related to clonal evolution after multiple treatments. Our study showed a high concordance (70%) between the primary and metastatic lesions in the 10 cases for which primary and metastatic tumors were available, with all primary tumors found to be AR+ and only three patients having AR- metastases. Since two of these three samples were cytology from pleural effusions, it is difficult to know whether the apparent loss of AR expression in all three cases represented true tumor evolution. However, our study, together with these others, has clinical relevance regarding the decision to biopsy metastatic lesions to determine AR status if anti-androgen therapy is being considered.

The main limitation of our study for determining the association between response to endocrine therapy and AR expression was the sample size of only 46 patients, with only 7 patients in the subgroup of 'poor' responders to endocrine therapy. An additional limitation was the high overall high prevalence of strong staining for AR with only 7 patients in the AR- subgroup and only 2 more in the 'weak' subgroup. For 36 patient's only primary or metastatic tumors but not both were available. The fact that samples from both primary tumor and one or more metastatic sites were only available for 10 patients, limited our ability to determine the true incidence of change in AR expression with tumor progression. Further studies in a larger and more selected population may be needed to verify our findings. Given the strong correlation between AR and ER, a case-control or prospective cohort study, intentionally selecting an approximately equal number of ER+ AR- and ER+ AR+ MBC patients and determining response to anti-estrogen therapy in each group, would be most enlightening.

In summary, AR expression is a common feature in ER+ tumors which is generally maintained in metastatic lesions. No relationship between AR expression or intensity and response to anti-estrogen treatment was found in our pilot study, although a larger sample may be necessary to confirm this finding. Given that AR is present in a high percentage of patients with ER+ MBC, better knowledge of the role of this pathway (if any) either concomitant with or after acquired resistance to other endocrine therapies is warranted, especially with the advent of new potent anti-androgen agents, as this may lead to attractive treatment opportunities.

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