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Original Research

Androgen receptor expression inversely correlates with immune cell infiltration in human epidermal growth factor receptor 2–positive breast cancer

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KEYWORDS
Androgen receptor expression;
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Abstract ***Introduction:*** Although targeting human epidermal growth factor receptor 2 (HER2) is a meaningful treatment in HER2-positive breast cancer, ultimately resistance develops. Androgen receptor (AR) expression and immune cell infiltration are thought to be involved in trastuzumab response and may, therefore, be of interest as additional targets for therapy in HER2-positive breast cancer.
Aim: To improve insights into the presence among AR expression, immune cell infiltration and HER2, we analysed HER2-positive breast tumours.
Methods: Primary tumours of 221 patients treated with trastuzumab for metastatic disease were selected. HER2 status was centrally confirmed. AR, T-cells (CD3 and CD8), programmed cell death protein 1 (PD-1) and PD-1 ligand 1 immunohistochemical staining and M2 tumour-associated macrophages (TAMs; CD68 and CD163) immunofluorescence were performed. Tumour-infiltrating lymphocytes were evaluated by haematoxylin and eosin staining.
Results: Sufficient tumour material was available for 150 patients. Oestrogen receptor was expressed in 51.3% of the tumours and AR in 81.3% of the tumours. AR expression was inversely correlated with M2 TAM (*Pearson's* $r = -0.361$, $P < 0.001$), CD3+ ($r = -0.199$, $P < 0.030$)

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and CD8+ ($r = -0.212$, $P < 0.021$) T-cell infiltration. Clustering analysis showed high immune cell infiltration in AR low-expressing tumours, and low immune cell infiltration in AR-high expressing tumours.
Conclusion: AR expression inversely correlates with immune cell infiltration in HER2-positive breast cancer.

1. Introduction

Human epidermal growth factor receptor 2 (HER2)–positive breast cancer accounts for 15–20% of all invasive breast cancers [1]. Trastuzumab-based anti-HER2 therapy added to chemotherapy improves overall survival (OS) of patients with HER2-positive breast cancer [2,3]. In the metastatic setting however, eventually resistance to trastuzumab regimens develops. Treatment strategies to counteract this, namely trastuzumab plus pertuzumab, lapatinib or antibody–drug conjugate T-DM1, have improved patients' survival [4–6]. However, eventually resistance to these approaches will develop [4,6]. Therefore, new targets for rational (combination) therapies are needed. Potentially, androgen receptor (AR) [7,8] and immune cell composition [9–11] are of relevance in this setting.

AR is a steroid receptor with important functions in cell differentiation and proliferation. In the presence of androgens, the ligand-bound AR binds to hormone response elements. This results in upregulation or downregulation of specific protein expression [12]. In patients with oestrogen receptor (ER)–negative/HER2-positive non-metastatic breast cancer, AR tumour expression has been associated with a trend for worse prognosis [13]. In preclinical models, targeting both HER2 and AR demonstrated a synergistic antitumour effect [7,8]. This is supported by preliminary results of an ongoing trial combining the AR signalling inhibitor enzalutamide and trastuzumab in breast cancer patients with HER2-positive/AR-positive tumours (NCT02091960). There was a 24-week clinical benefit observed in six of the 18 patients receiving more than four prior lines of therapy [14].

The immune cell composition of the breast cancer environment has been related to prognosis of patients, depending on the tumour molecular subtype [15,16]. Moreover, preclinical and clinical evidence suggests that immune cell compositions are able to predict response of in HER2-targeted treatment [9–11]. Presence of CD8+ T-cells in tumours predicted a better response to HER2-targeted treatment in preclinical mouse models [10]. However, an immunosuppressive microenvironment, such as presence of tumour-associated macrophages (TAMs) or programmed cell death protein 1 (PD-1)–expressing immune cells, contributed to HER2-targeted treatment resistance in similar mouse models [11,17]. These data provided the rationale for combining trastuzumab with the immune checkpoint inhibitor pembrolizumab in 46 patients with HER2-positive tumours, which resulted in a modest 15% objective response rate [18]. This indicates that patient selection for this combination therapy should be improved. Increased knowledge of immune microenvironment composition in HER2-positive breast cancer could possibly be of relevance in this respect.

Therefore, to improve insights into the interaction among AR expression, immune cell infiltration and HER2, we analysed in primary HER2-positive breast tumours the AR expression and infiltration of M2 TAMs, CD3+ and CD8+ T-cells, PD-1+, PD ligand 1+ (PD-L1+) cells and tumour-infiltrating lymphocytes (TILs). These findings were related to OS.

2. Material and methods

2.1. Study population and breast tumour samples

Tissue microarrays (TMAs) containing primary tumour material from a retrospectively collected cohort of 221 patients with HER2-positive metastatic breast cancer were used. Patients treated with trastuzumab and concurrent chemotherapy of physicians' choice, for metastatic breast cancer, were identified in the records of 19 hospital pharmacies in the northern part of the Netherlands. Details of patient selection, patient and treatment characteristics, follow-up and TMA construction have been described previously [19,20]. Per tumour, three cores were incorporated in the TMA. Patient, treatment and tumour characteristics, including presence in the primary tumour of expression of ER and progesterone receptor (PR), with 10% of cells expressing ER or PR used as cut-off for positivity, were collected from the Netherlands Cancer Registry. HER2 status was centrally reviewed as described previously [19]. According to the Dutch Central Committee on Research involving Human Subjects, this retrospective non-interventional study did not require approval from an ethical committee in the Netherlands. This study was approved by the Privacy Review Board of the Netherlands Cancer Registry.