

Is the skin a sanctuary for breast cancer cells during treatment with anti-HER2 antibodies?

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Abbreviations: ADCC, Antibody-Dependent Cell-mediated Cytotoxicity; APCs, Antigen Presenting Cells; HER2, Human Epidermal Growth Factor Receptor 2; HR, hormonal receptors; IDC, invasive ductal carcinoma; T-regs, regulatory T cells; TLR, Toll-like receptor; TDM-1, Trastuzumab Emtansine

The occurrence of skin metastases is a common event in patients affected by advanced breast cancer, usually associated with systemic disease progression. Here we describe 2 cases of diffuse cutaneous metastases from HER2-overexpressing breast cancer occurring despite a dramatic response in liver and bone, respectively, during treatment with anti-HER2 antibodies Trastuzumab and Pertuzumab. We discuss the reasons for this discrepancy and suggest a possible implication of impaired immune response in the skin. Future research should provide strategies to overcome the induction of immune privilege in the skin in order to avoid discontinuation of effective treatments.

Introduction

Dual blockade of Human Epidermal Growth Factor Receptor 2 (HER2), obtained with the monoclonal antibodies Pertuzumab and Trastuzumab in combination with Taxanes is now considered the best option for first line treatment of HER2-overexpressing metastatic breast cancer. Cleopatra trial has recently shown a significant increase in progression free survival and overall survival when Pertuzumab is added to a standard treatment including Trastuzumab and Docetaxel.¹ This advantage has also been observed in patients who recurred after neoadjuvant/adjuvant Trastuzumab containing regimens, even if a definitive conclusion on this issue is limited by small sample size.²

However, the addition of Pertuzumab does not reduce the percentage of brain metastasis, a site usually considered a sanctuary where antibodies have limited efficacy.³ The reasons for this failure are generally attributed to the presence of the brain-blood barrier that strongly protects the nervous system from several drugs including monoclonal antibodies,^{4,5} or to the so called "immune privilege" of the brain,^{6,7} i.e. the lack of an adequate immune response in part required for monoclonal antibody effectiveness. This latter aspect is particularly important for Trastuzumab and Pertuzumab that, more than other antibodies, have been shown to function through Antibody-Dependent Cell-mediated Cytotoxicity (ADCC).^{8,9}

Interestingly, immune privilege has been described also in the skin.^{10,11} The exact mechanisms involved have been only

partially elucidated, including (i) lack of MHC class II expression in Antigen Presenting Cells (APCs) determining impaired immune function in the inferior portions of hair follicles,¹⁰ (ii) production of potent immune-suppressants, such as TGF- β 1 and α -melanocyte-stimulating hormone (α -MSH),¹⁰ or (iii) upregulation of the negative co-signaling molecule PD-L1 in dermal papilla and dermal sheath cup cells.¹¹

Several cases of cutaneous progression after or during Trastuzumab regimens have been described in the literature, frequently associated with systemic disease progression.^{12,13}

Herein we describe 2 cases in which patients with HER2-positive metastatic breast cancer had loco-regional cutaneous progression during treatment with Pertuzumab and Trastuzumab despite an impressive systemic response.

We hypothesize that cancer cells located into the skin would survive and take proliferative advantage in virtue of an immune-tolerance mechanism that hampers trastuzumab-mediated ADCC.

Case presentation

Case 1

A 62-year-old woman came to our attention in October 2011 after she noticed a lump in her left breast. Clinical examination revealed a 7x5 cm mass with signs of inflammatory breast cancer. Bilateral breast MRI confirmed a mass-like lesion suggestive of

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malignancy in the left breast. A tru-cut biopsy was performed and histopathology showed an invasive ductal carcinoma (IDC) with HER2-overexpressing phenotype: hormonal receptors (HR) negative, HER2 positive (3+), Ki67 30%.

A sequential scheme of Epirubicin plus Cyclophosphamide for 4 cycles followed by Docetaxel plus Trastuzumab for 4 cycles was administered as neoadjuvant therapy. A partial clinical response was obtained and total mastectomy with complete axillary node dissection was performed. Pathology revealed a residual cancer of 3 mm in the breast and the presence of metastases in 2 nodes. Immunohistochemistry confirmed the initial biology of the tumor. Staging was ypT1a, ypN1a, cM0.

She further underwent radiotherapy to the left chest wall and supraclavicular fossa, and received adjuvant Trastuzumab to complete 1 year. After 5 months, several red plaques appeared on her left chest skin, close to the surgical scar. Skin biopsy showed cutaneous infiltration of a highly proliferative (Ki67 70%)

invasive ductal carcinoma of mammary origin. Neoplastic cells were negative for HR and strongly positive for HER2. Since there were no radiological signs of metastatic disease, a wide cutaneous excision was performed.

Unfortunately, 3 months later, red cutaneous plaques reappeared on her skin, in the site of previous excision, and a restaging CT scan showed secondary lesions in the liver (Fig. 1A-B). A first line chemotherapy with Pertuzumab, Trastuzumab and Docetaxel was started. A clinical objective response was achieved on the cutaneous lesions during the treatment and a CT scan performed after 5 cycles revealed a partial response in liver (Fig. 1C vs A and D vs B). Liver lesions gradually continued to reduce up to a complete radiological response after 12 cycles of treatment (Fig. 1E vs A and F vs B). At that time, paradoxically, red patches started to appear on her contralateral breast, similar to urticarial reaction. Patient's right breast rapidly became oedematous and covered by little nodules resembling the plaques of the left chest wall that, in the meantime, increased in size and number, a clear sign of cutaneous cancer progression (Fig. 2).

A new CT scan confirmed that the patient was without any evidence of systemic progression and maintained a complete radiological response in the liver. She discontinued Pertuzumab plus Trastuzumab and started a new therapy with Trastuzumab Emtansine (TDM-1).

Case 2

A 50-year-old woman was admitted to our Breast Unit because of a typical appearance of inflammatory cancer in the left breast with small, well-circumscribed, solitary nodules in the trunk and in the base of the neck. Her left breast appeared swollen, red and oedematous. Bilateral mammography showed a 3 cm mass in the left breast with increased skin thickness. Mammogram was normal in the right breast. Biopsy confirmed the presence of lymphangitis carcinomatosa sustained by HER2-overexpressing, HR-negative IDC. Staging CT scan showed no sign of systemic disease and she started neoadjuvant therapy with Epirubicin plus Cyclophosphamide according to a dose-dense schedule (every 2 weeks).

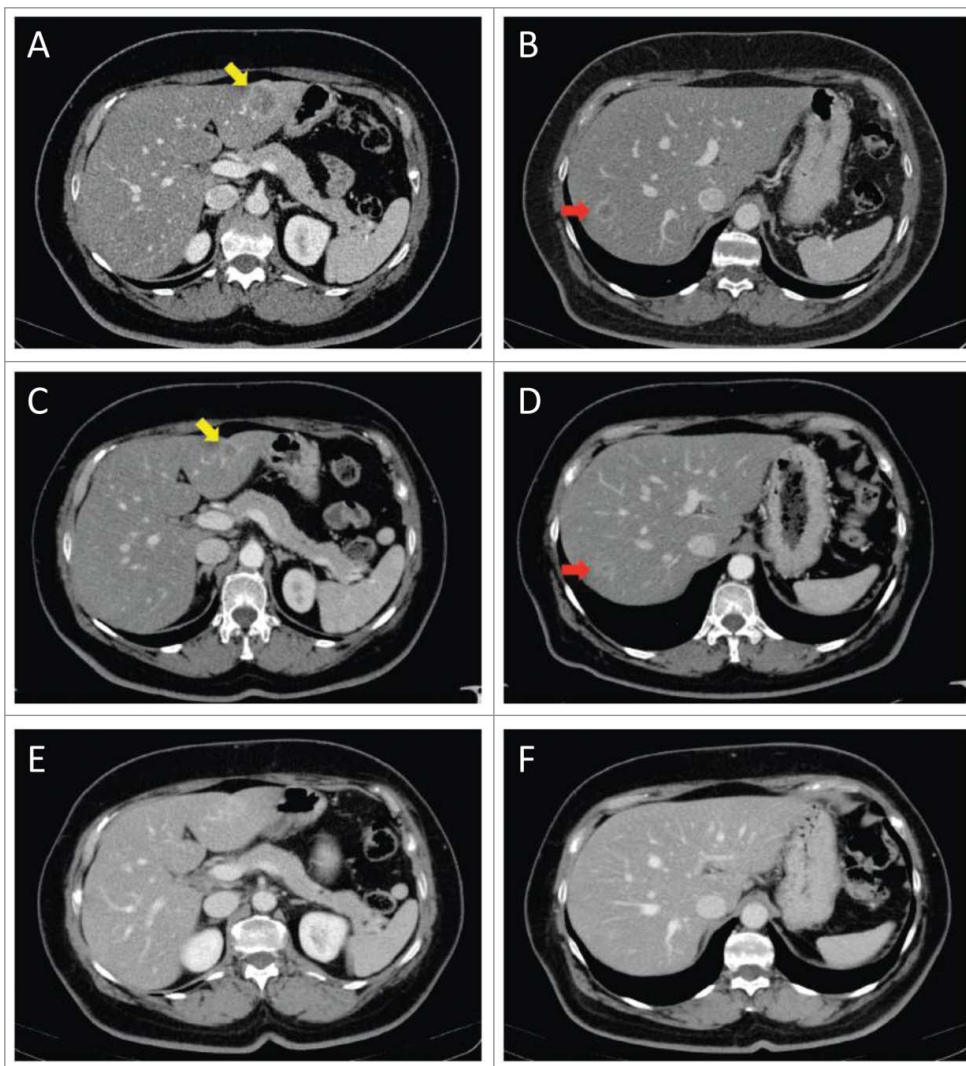


Figure 1. Response in patient 1. CT scan shows liver metastasis presentation at basal time (A and B), after 5 cycles (C and D) and after 12 cycles (E and F) of chemotherapy containing Trastuzumab/Pertuzumab. The target lesions are indicated by arrows.



Figure 2. Skin metastases during Trastuzumab/Pertuzumab treatment in patient 1. The red patches on the right oedematous breast resemble urticarial reaction. Nodules and plaques spread all over the left chest wall.

After four cycles she complained of pain in her right hip. Bone scan revealed the presence of multiple metastases in the spine and pelvis. MRI confirmed neoplastic lesions in all vertebra and pelvic bones (**Fig. 3A**). She underwent radiotherapy to the proximal part of her right femur and subsequently initiates first line therapy with Trastuzumab, Pertuzumab and Docetaxel. She rapidly reached clinical benefit: the inflammatory signs of the left breast gradually faded and the skin nodules decreased. Consistently, the MRI performed after 6 cycles demonstrated a dramatic reduction in number and size of bone metastases (**Fig. 3B**).

After 9 months of treatment (12 cycles) she noticed reddening of her trunk and a swollen, oedematous right breast, the contralateral one, resembling a cutaneous infection. Topical steroids, oral nonsteroidal anti-inflammatory drugs (NSAIDs) and antibiotics were prescribed without any benefit. A biopsy of the para-

areolar skin was performed and histological examination disclosed a HER2-overexpressing IDC. New small nodules appeared in the skin of the trunk and the pre-existing ones increased in size (**Fig. 4**). Nevertheless, disease remained in partial response in the bone and with no other site of metastatic spread as revealed by MRI (**Fig. 3C**) and CT scan, respectively. She discontinued treatment and started a second line therapy with TDM-1.

Discussion

To date, the contemporary use of 2 anti-HER2 antibodies, Pertuzumab and Trastuzumab, in association with Taxanes represents the best therapy for HER2-positive metastatic breast

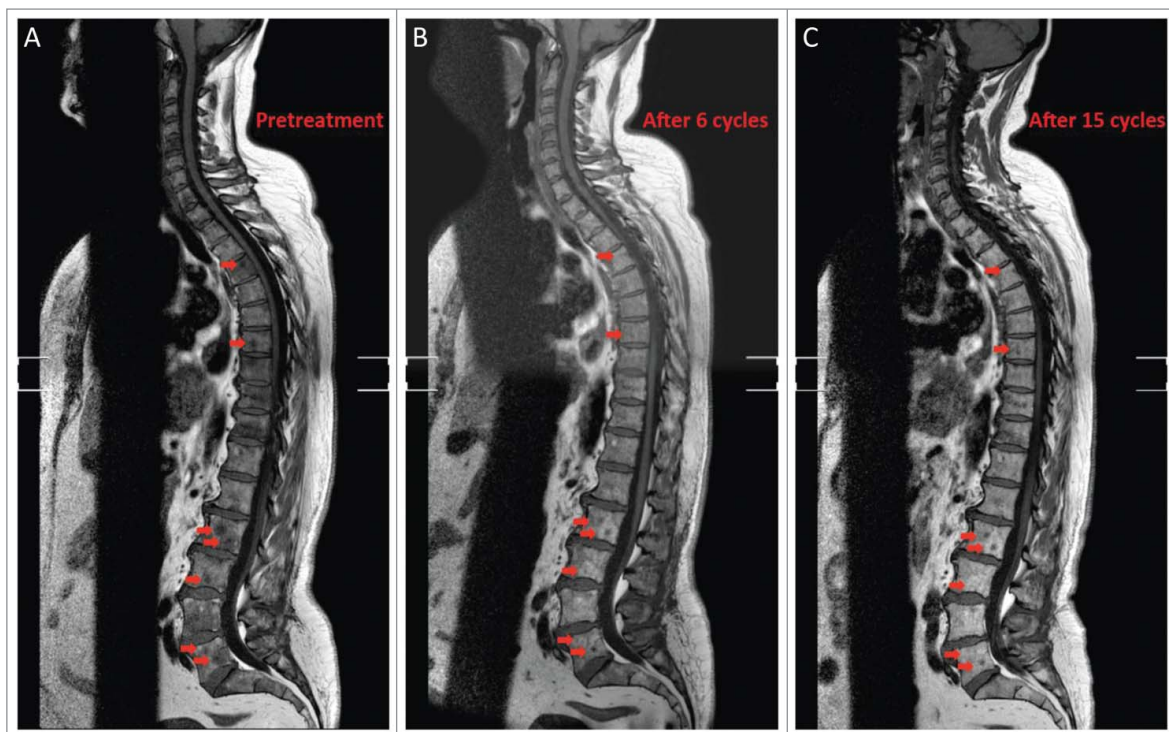


Figure 3. Response in patient 2. RMI shows several metastatic lesions in all vertebra at basal time (**A**), after 6 cycles (**B**) and after 12 cycles (**C**) of chemotherapy containing Trastuzumab/Pertuzumab. Arrows indicate some target lesions.



Figure 4. Skin metastases during Trastuzumab/Pertuzumab treatment in patient 2. Edema of the right breast and reddening of the skin on the trunk up to the base of the neck with the presence of small, well-circumscribed, solitary nodules. Biopsy confirmed the malignant origin of the lesions.

cancer, and it is probably intended to become the best option in several lines of treatment. A remarkable median overall survival of almost 5 years has been reported for therapy-naïve metastatic patients.^{1,2}

The two cases described herein are disappointing for the failure of Pertuzumab/Trastuzumab treatment because of diffuse cutaneous cancer progression in spite of an important systemic control of the disease. Skin metastases are rare events among all cancers, but develop in 24% of cases in advanced breast cancer,¹⁴ especially if HER2 overexpressing.¹⁵ Both our cases were inflammatory breast cancer, a biologically different type of neoplasm characterized by a high risk of local-regional recurrence, estimated around 20% even after a multimodal treatment including chemotherapy, surgery and radiotherapy, and at high risk of distant metastases.¹⁶ Once metastatic, the treatment of inflammatory cancer does not substantially differ from that of other types of breast cancers, even if the prognosis remain poor.

The appearance of reddening, oedematous area in the skin has been observed more frequently in patients treated with Pertuzumab plus Trastuzumab compared to Trastuzumab alone.¹ In some cases the rush impose to investigate differential diagnosis between skin metastases and bacterial colonization sustained by *Staphylococcus* species.¹⁷ Biopsy is recommended in absence of clinical improvement after antibiotic and/or anti-inflammatory therapies. Our second patient had cutaneous manifestation resembling skin infection, but no clinical change was noticed after specific antibiotic treatment and the subsequent biopsy confirmed a malignant origin of the skin lesions.

In breast cancer patients cutaneous metastases are often associated with systemic progression of the disease,¹⁸ generally related to the selection of a more aggressive, resistant clone. Paradoxically, our 2 patients developed rapid progressive cutaneous metastases while achieving an impressive response in liver and bone, respectively.

Several mechanisms of resistance to Trastuzumab have been described in preclinical model,¹⁹ including epitope masking by mucin-4 (MUC4) or CD44 overexpression,^{20,21} expression of p95Her2, a truncated form of the HER2 protein,²² upregulation of HER2 downstream signaling pathways by loss of PTEN or PI3K mutations,^{23,24} and alteration of ADCC.²⁵

Although the unique cancer progression in the skin might be due to the selection of a resistant clone according to the above mentioned mechanisms, we hypothesize a change in the tumor cutaneous microenvironment leading to the establishment of a sanctuary-like site in which tumor cells can easily escape from anti-HER2 directed antibodies. As a blood barrier that limits cutaneous distribution of antibodies has never been described, we hypothesize a process of acquired immune tolerance in the skin that impairs the ADCC triggered by Pertuzumab/Trastuzumab. This assumption comes from the following considerations.

First, impaired trastuzumab-mediated ADCC has been reported as a mechanism of resistance to Trastuzumab.^{19,25} Second, the selection of a resistant clone with an exclusive tropism for the skin, although possible, has never been described in breast cancer. Third, a huge systemic response with a parallel diffuse skin progression strongly suggest that the "soil," i.e the skin, rather than the "seeds," i.e., metastatic clones, is involved in this process. Fourth, the simultaneous progression in the skin of multiple pre-existing nodules, distant one from each other, is an evidence of an alteration in the drug/cancer cells/microenvironment interaction. It is hard to believe that a clonal selection takes place in different sites at the same time. Finally, the skin has been described as a site in which active mechanisms of immune tolerance can occur. Evidences have been provided showing that chronic infections²⁶ or immunological tolerizing procedures^{27,28} can induce immune tolerance/privilege specific for skin tissue. The activation of regulatory T cells (T-regs) seems to be responsible for this immunosuppressive process.^{29,30}

The idea that a local impaired immune system may promote skin metastases progression is supported by the evidence that topical imiquimod, a Toll-like receptor (TLR)-7 agonist, is active in the treatment of skin metastases from different neoplasms, including breast cancer,³¹⁻³³ basal cell carcinoma³⁴ and melanoma.³⁵ TLRs are known to suppress the function of T-regs by breaking tolerance and enhancing immune responses against cancers.³⁶⁻³⁸

Understanding the mechanisms of induced immune privilege in the skin, including those occurring during Pertuzumab/Trastuzumab treatment, is essential for the development of new therapeutic strategies able to restore anti-tumor immune response. In

this way, an effective systemic therapy such as Pertuzumab/Trastuzumab could be continued while controlling skin metastasis.

At our knowledge these are the first 2 cases of Pertuzumab-containing regimen discontinuation for progression of isolated cutaneous metastases despite a dramatic response in other metastatic sites, such as liver and bones. We hope future clinical trials could provide strategies to avoid giving up extremely efficient treatments, potentially curative for some patients, in a non-life-threatening condition like skin metastases.

Authors' contributions

VG, MTS, MZ, JG and AG: treated the patients and collected the data; VG and AG reviewed the literature and wrote the paper;

VG, AG, PV, CN, carried out critical interpretations. All authors read and approved the final manuscript.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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