

p185^{HER2} overexpression and *HER2* oncogene amplification in recurrent vulvar Paget's disease

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We evaluated p185^{HER2} overexpression and *HER2* oncogene amplification in recurrent vulvar Paget's disease. We identified six patients with recurrent vulvar Paget's disease in our archives. The number of recurrences ranged from 1 to 11 over a time period of 1–168 months. Recurrences were evaluated immunohistochemically for p185^{HER2} overexpression with the HercepTest[®] and for *HER2* oncogene amplification with fluorescence *in situ* hybridization. p185^{HER2} overexpression was scored as 3 in two patients, as 2 in two patients, and as 1 in two patients. All patients with scores 2 and 3 showed *HER2* oncogene amplification. Overexpression of p185^{HER2} and *HER2* oncogene amplification appears to be common in recurrent vulvar Paget's disease.

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Paget's disease of the vulva is an uncommon carcinoma of apocrine gland origin. Most cases are limited to the epidermis and mucosa, but some invade the dermis. Definitive treatment of vulvar Paget's disease is often difficult because of the anatomical and surgical limitations in this region. Surgical excision is associated with recurrence rates of up to 60%.^{1–3} This is often due to positive resection margins, but it has also been suggested that Paget's disease arises multifocally from pluripotent stem cells.

Human epidermal growth factor receptor 2 (*HER2*) oncogene, encoding for the 185-kDa transmembrane glycoprotein receptor (p185^{HER2}), is involved in transformation and progression of many human cancers, including Paget's disease.^{4–9} Tanskanen *et al*⁵ reported *HER2* oncogene amplification and p185^{HER2} overexpression in seven of 13 primary vulvar Paget's disease, but p185^{HER2} expression in recurrent Paget's disease has not been studied. We analyzed p185^{HER2} overexpression and *HER2* oncogene amplification in six patients with recurrent vulvar Paget's disease.

Materials and methods

Among the 11 patients with primary Paget's disease identified in the archives of our institutions, six had recurrent disease. All vulvar tissues of the primary and recurrent vulvar Paget's disease were analyzed immunohistochemically with HercepTest[®] (DAKO, Denmark), an FDA-approved assay of p185^{HER2} overexpression. Staining results were scored as score 0 (negative; no staining is observed, or membrane staining in less than 10% of the tumor cells); score 1 (negative; faint/barely perceptible focal membrane staining in more than 10% of tumor cells); score 2 (positive; weak to moderate staining of the complete cell membrane in more than 10% of the tumor cells) and score 3 (positive; strong staining of the complete membrane in more than 10% of the tumor cells). Positive and negative controls were used to validate each run of the assay. All cases with a score 2 and 3 were further analyzed with fluorescence *in situ* hybridization (FISH, PathVysion *HER2*-DNA probe kit; Vysis, Downers Grove, IL, USA).

Results

All six patients with recurrences had positive surgical margins in the initial and recurrent excisions (Figure 1). Recurrences were seen as early as 1 month after primary surgery. Two patients had one recurrence, two patients had two, and one patient

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had three recurrences. One patient had 11 resections of recurrent disease over a period of 168 months after primary vulvectomy (Table 1). Three patients developed invasive disease in their recurrences after 9, 58 and 168 months, respectively (pt. 1, 4 and 5). p185^{Her2} overexpression was scored as 3 in two patients, as score 2 in two patients, and as score 1 in 2 patients (Figure 2a–d). All patients with score 2

and score 3 showed *HER2* oncogene amplification (Figure 3). The recurrences showed the same scores as the corresponding primary tumors (Table 1). Analysis of the five tumors that did not recur showed a score 3 and *HER2* oncogene amplification in three patients, while two patients were negative for p185^{Her2} overexpression.

Discussion

This is the first report of p185^{Her2} overexpression and *HER2* oncogene amplification in recurrent Paget's disease of the vulva. In a large retrospective series of 100 women with Paget's disease treated in eight institutions, 34% of patients developed recurrences in the median time of 3 years.¹ This is partly due to the apparently multifocal nature of the disease and partly to the difficulties in recognizing the extent of the lesions clinically. Recurrences have also been described in skin grafts from other parts of the body.¹⁰ The mechanism by which Paget's cells spread intraepidermally and infiltrate the dermis seems to be induced by a mobility factor (heregulin-alpha) that acts through the p185^{Her2} receptor.¹¹ The major problem with the recurrences was the extension of the Paget's disease to surrounding nongenital skin such as the inner side of the thigh, perianal skin and mons pubis, rendering surgical revision excision difficult.

Trastuzumab is a recombinant DNA-derived humanized human monoclonal immunoglobulin G (IgG) antibody that binds to the antigen of p185^{Her2}. Immunotherapy with trastuzumab is a well-tolerated option for first-line treatment of women with metastatic breast cancer and presently under investigation for several other cancers.¹² Possible antiproliferative mechanisms of trastuzumab include downregulation of *HER2* receptors, activation of immune effector cells (antibody-dependent cell-mediated cytotoxicity), reduction of S-phase cell-cycle progression, and reduction of vascular endothelial growth factor for angiogenesis.¹³ However, trastuzumab is generally confined only to patients



Figure 1 Recurrent vulvar Paget's disease involving the entire vulva, perineum and perianal skin and spreading to skin of inner thighs of a 63-year-old woman (pt. #1). Note that the Paget's disease extends well beyond the indicated surgical margins.

Table 1 Characteristics of six women with recurrent vulvar Paget's disease

Patient	Age at primary treatment (years)	Primary treatment	Surgical margins	Time to first and last recurrence (months)	Number of recurrences	HER2			
						Primary tumor		Recurrence	
						IHC	FISH	IHC	FISH
#1	62	Surgery	Pos	6/9	2	3+	Ampl	3+	Ampl
#2	71	Surgery	Pos	26	1	1+	—	1+	—
#3	57	Surgery	Pos	4	1	1+	—	1+	—
#4	51	Surgery	Pos	24/168	11	2+	Ampl	2+	Ampl
#5	63	Surgery	Pos	46/58	2	3+	Ampl	3+	Ampl
#6	75	Surgery	Pos	1/52	3	2+	Ampl	2+	Ampl

IHC, immunohistochemistry; FISH, fluorescence *in situ* hybridization; pos, positive; Ampl, Amplification.

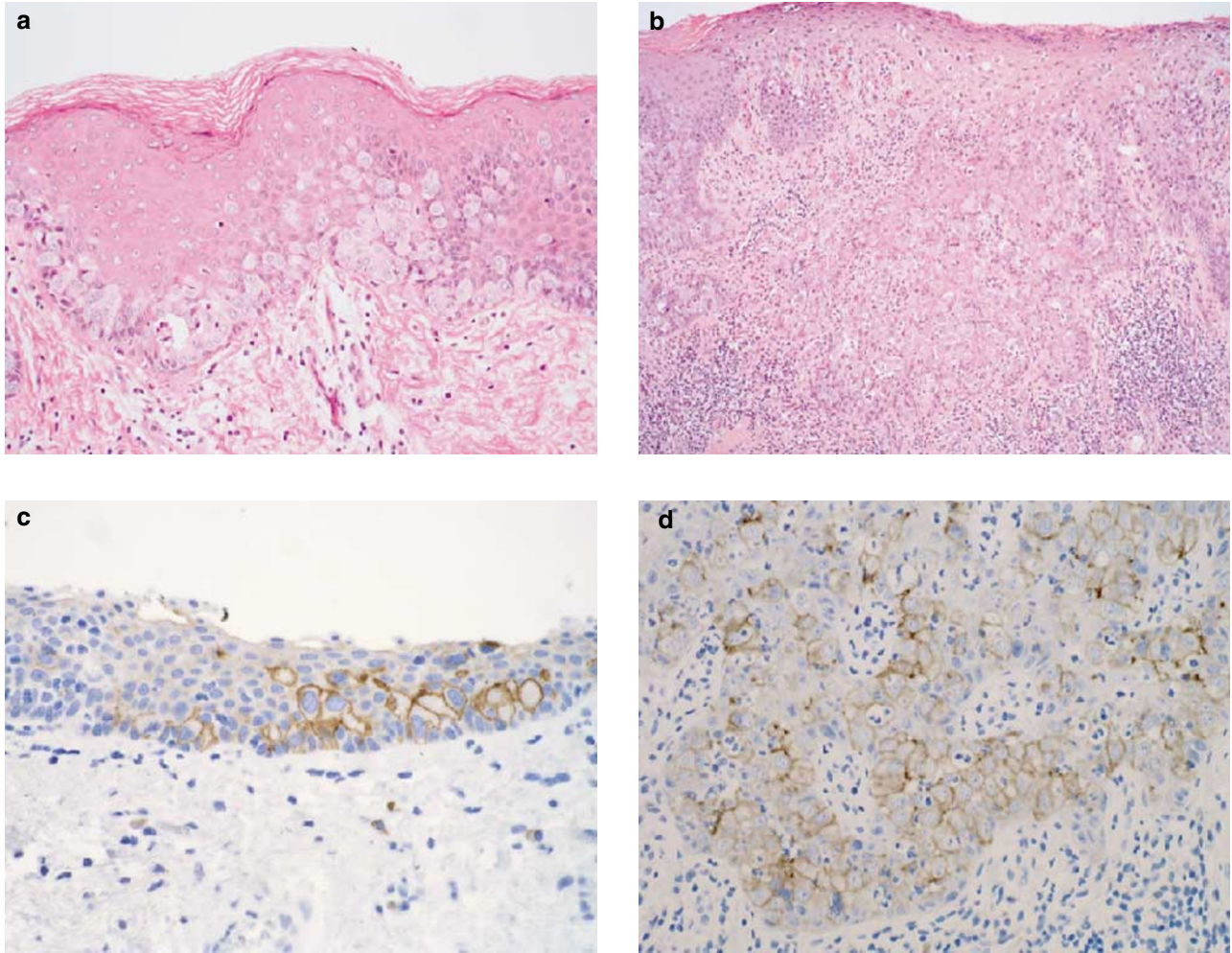


Figure 2 Hematoxylin and eosin stains of the recurrent Paget's disease with intraepidermal proliferation of large pale cells (a) and focal invasion (b) (pt. #4); (c) p185^{Her2} overexpression in intraepidermal Paget's cells with a strong membranous staining of the entire cell membranes, score 3 (pt. #1, see clinical picture in Figure 1); (d) p185^{Her2} positivity (score 2) in an invasive area of recurrent Paget's disease (pt. #4).

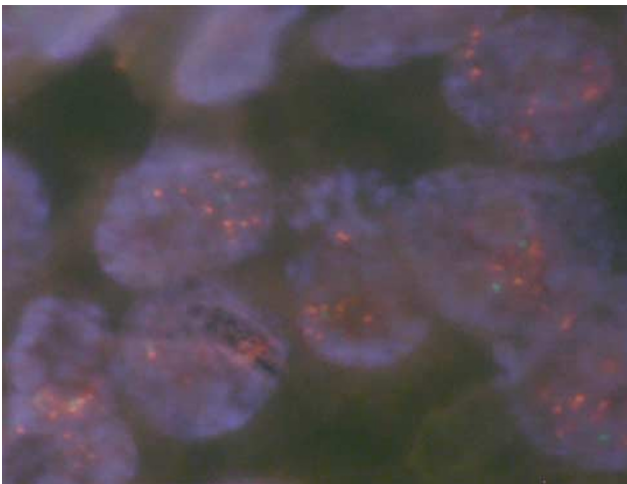


Figure 3 FISH analysis for *HER2* oncogene amplification in vulvar Paget's disease (pt. #1). Red signals indicate *HER2* oncogene amplification, green signals correspond to CEP 17.

whose tumors stain at score 3 level or with gene amplification detected by FISH.

In conclusion, overexpression of p185^{Her2} and *HER2* oncogene amplification appears to be common in primary and recurrent vulvar Paget's disease including the invasive components. These results suggest that a clinical trial of trastuzumab for women with high-risk Paget's disease of the vulva is justified.

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