Histopathologic and Immunohistochemical Characterization of Rash to Human Epidermal Growth Factor Receptor 1 (HER1) and HER1/2 Inhibitors in Cancer Patients

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Abstract

Purpose: Human epidermal growth factor receptor (HER) 1 and HER 1/2 inhibitors have shown benefit against a wide range of solid tumors. However, their use is associated with rash in 40% to 90% of patients, which impacts quality of life and interrupts antineoplastic therapy. The pathologic characteristics of affected skin remain unclear, precluding development of rational therapies. The aim of this study was to evaluate differences in histologic and immunohistochemical alterations in rash caused by lapatinib, a dual HER1/2 inhibitor (HER1/2i), and the single HER1 inhibitors (HER1i) cetuximab, erlotinib, and panitumumab.

Experimental Design: For each of the four drugs, skin biopsies were collected and analyzed from 8 patients with rash (n = 32). Blinded independent histologic analysis and automated measurement of 17 skin biomarkers involved in proliferation, differentiation, and inflammation were conducted.

Results: Increased expression of pAKT and decreased dermal K16 and p27 for HER1/2i when compared with each of the HER1i were observed. In addition, decreased epidermal atrophy and follicular neutrophilic infiltrate were evidenced in the skin of patients on HER1/2i when compared with HER1i.

Conclusions: We found a lower inhibition of epidermal kinetics and decreased inflammation in HER1/2i-induced rash. These findings underscore differences in skin toxicity as related to specificity of HER blockade, concordant with clinical tolerability and decreased severity of skin toxicity seen with the HER1/2i lapatinib compared with the HER1 inhibitors cetuximab, erlotinib, and panitumumab.

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Human epidermal growth factor receptor inhibitors (HER1i) have shown effectiveness against a wide variety of solid tumors. In particular, they are used in malignancies overexpressing HER 1 and 2, including head and neck, breast, lung, pancreatic, and colorectal cancers (1, 2). To date, regulatory agencies have approved agents that are single HER1 inhibitors (i.e., cetuximab, erlotinib, and panitumumab) and a dual inhibitor targeting both HER1 and HER2 (lapatinib). The most clinically significant toxicity associated with the use of HER1 is a papulopustular (also referred to as acneiform) rash that affects the face and upper body and develops in up to 90% of patients.

This rash has a negative impact as it relates to quality of life (3), cost (4), secondary infections (5), and the ability to maintain antineoplastic therapy without interruption (6), all of which may also affect clinical outcome. In skin, HER1 is primarily expressed in basal and suprabasal keratinocytes, sebocytes, and the outer root sheath of the hair follicle (7). Activation of HER1 by its ligands, epidermal growth factor (EGF), transforming growth factor-α, amphiregulin, and heparin-binding EGF, has been shown to regulate keratinocyte proliferation, differentiation, migration, and survival (8). HER1-driven proliferation results in downstream activation of phosphatidylinositol 3-kinase (PI3K)-Akt and mitogen activated protein kinase pathways regulating keratinocyte survival, proliferation, and differentiation.

Blockade of HER1 in skin induces apoptosis in normal keratinocytes, which increases 5-fold between day 4 and 12, and which correlates with median time to rash onset in patients (9). Increased chemokine expression after HER1 blockade has been shown to be regulated by extracellular regulated kinase 1 and 2 (ERK1/2), resulting in enhanced skin inflammation (10). HER1 inhibition induces early differentiation by upregulating the expression of terminal differentiation markers, such as keratin1
Human epidermal growth factor receptor (HER) 1 and 1/2 inhibitors are widely used in a variety of solid tumors. The use of these drugs frequently results in a rash that may lead to dose modification and decreased quality of life. In spite of coincident targets, the dual HER1/2 inhibitor (HER1/2i) lapatinib has a lower incidence of rash, when compared with the single HER1 inhibitors (HER1i) cetuximab, erlotinib, and panitumumab. We studied the histologic and immunohistochemical alterations that may provide insight into the clinical variability of rash severity. A decrease in epidermal atrophy and neutrophilic follicular infiltrate, along with higher pAKT and lower p27 and K16 expression were seen with HER1/2i, when compared with HER1i. Histologic and immunohistochemical findings for lapatinib correlate with clinical observations of decreased skin toxicity.

(KRT1) and KRT10 (11). In addition, increased signal transducers and activators of transcription 3 (STAT3) staining in the basal layer of the epidermis (12) occurs, indicative of premature differentiation. Decreased expression of cytoskeletal proteins, f-actin-binding protein vinculin and the actin-binding protein actinin-α1 (ACTN1), resulting in decreased motility (13) ensues, along with increased attachment via cadherin-associated protein catenin-δ2 (CTNND2) and desmoglein 2 (DSG2; ref. 14; Supplementary Data).

Clinically, HER1/2 inhibition by lapatinib results in a lower incidence of rash (41%; ref. 15), when compared with the HER1 inhibitors erlotinib, cetuximab, and panitumumab (75-90%; ref. 16–18). Based on rash severity, patients on HER1/2i are less likely to require dose modifications when compared with HER1i. The mechanisms responsible for this differing skin toxicity profile remain unknown, as preclinical data show a similar inhibitory profile for these agents. This study investigated the histologic and immunohistochemical characteristics that may explain some of the clinical differences in rash between HER1 inhibitors (HER1i) and the HER1/2 inhibitor (HER1/2i).

Materials and Methods

Patients

Upon Institutional Review Board (IRB) approval, the existing medical records including archived skin biopsy specimens and dermatopathology reports of patients with rash attributed to treatment with lapatinib, cetuximab, panitumumab, or erlotinib were analyzed. All patients were seen between January 2006 and December 2007. A total of 8 samples per patient/inhibitor were collected, with a total of 32 patient specimens analyzed for this study. Anatomic site of skin biopsy specimens was variable and based on the location of rash, with a majority located on the upper trunk.

Immunohistochemistry

For each subject, immunohistochemical studies were done on 5-μm sections of formalin-fixed parafin-embedded tissue by using an Envision kit (Dako), a peroxidase-conjugated polymer detection system, and diaminobenzidine (DAB) as chromagen on a Dako autostainer. An automated cellular imaging system (ACIS II, ChromaVision Medical Systems, Inc.) was used to quantify the staining of each molecular marker. The ACIS II software also calculates the average percentage and intensity of stained cells. Positive staining was calculated by applying two thresholds, with one recognizing blue background (hematoxylin stained) on cells and the other recognizing brown (DAB) positive cells. The percentage of positivity was the area detected by the brown threshold divided by the sum of the area detected by the brown and blue thresholds. The intensity was calculated by masking out all areas not selected by the brown threshold and calculating the integrated optical density of brown within the remaining area. This value was divided by the area in pixels of the brown mask to calculate an average intensity of a selected area (19). Seventeen immunohistochemical biomarkers were used for each case, and they included KRT1, HLA-DR, ERK1, Ki67, p27, pAKT, HER1, pHER1, CD68, CD54, CD20, CD11b, CD4, CD8, CD1a, and STAT3 (Table 1).

Histopathology

Each biopsy was a 4-mm archived skin specimen obtained for research purposes with Northwestern University IRB approval. Following formalin fixation and paraffin embedding, staining with H&E was done and specimens were submitted to two dermatopathologists (P.G. and J.G.) for independent blinded assessment of the epidermis, dermis, follicle, and inflammatory infiltrate. Biopsies were separately evaluated for the presence of epidermal, dermal, follicular, eccrine gland, and sebaceous gland alterations. Specifically, the epidermis was evaluated for the presence of ulceration, parakeratosis, acanthosis, epidermal atrophy, dysmaturation, dyskeratosis, and infiltrates of neutrophils, monocytes, or eosinophils. The dermis was evaluated for the presence of neutrophilic, monocytic, or eosinophilic infiltrates. The follicle was evaluated for bacterial colonies/concretions, neutrophilic pustules, dysmorphic features, dyskeratosis, and neutrophilic, monocytic, and eosinophilic follicular infiltrates. Finally, eccrine and sebaceous glands were evaluated for inflammatory infiltrates; eccrine glands were also evaluated for necrosis and dyskeratosis. Histologic features were rated as 0 (absent) or 1 (present), with the exception of infiltrate, rated from 0 (absent) to 3 (most prominent). If a specific structure such as a follicle or eccrine gland was not present in the specimen, the case was not included in the statistical analysis.
Statistical analysis
Seventeen immunohistochemical biomarkers and 23 dermatopathology features were statistically analyzed for the 8 specimens in each of 4 drug treatment groups. The biomarkers were continuous and analyzed using ANOVA methods. For each specimen positive staining calculations were determined. A two-factor nested repeated measures ANOVA was used, with drug as the between-subject factor and subject nested within drug and skin layer (epidermis or dermis) as the within-subject factor. A drug by skin layer interaction term was included in this analysis to determine whether the pattern of differences in means across drugs differed by skin layer. In addition, P values for main effects of drug and of skin layer are reported. Within each layer, a one-way ANOVA was carried out to determine differences across drugs. A P value comparing the dual HER1/2i (lapatinib) with all other single HER1i combined was also reported. Pairwise comparisons were done across drugs using independent sample t-tests without correction for multiple comparisons. In all these analyses, separate variances were estimated for each drug-skin layer combination. Histopathologic features were compared across groups using Fisher’s exact test. P values <0.05 were considered statistically significant.

Results

Patients
A total of 32 specimens were analyzed, 8 for each HERi. Complete demographic data for these patients is shown in Table 2. For patients on erlotinib, rash severity was grade 1 (n = 5; 63%), grade 2 (n = 1; 13%), and grade 3 (n = 2; 25%); for cetuximab, severity was grade 1 (n = 4; 50%), grade 2 (n = 3; 37.5%), and grade 3 (n = 1; 12.5%); for panitumumab, severity was grade 1 (n = 2; 25%), grade 2 (n = 3; 37.5%), and grade 3 (n = 3; 37.5%); and for lapatinib, severity was grade 1 (n = 3; 37.5%), grade 2 (n = 4; 50%), and grade 3 (n = 1; 12.5%).

Dual HER1/2 inhibition is associated with increased pAKT, and decreased K16 and p27 in skin
Significant immunohistochemistry results are summarized in Fig. 1. When comparing HER1/2i with the three HER1i, immunohistochemical analysis revealed a significantly increased expression of pAKT in both epidermis (P = 0.043) and dermis (P = 0.007), and a decreased expression of K16 (P = 0.032) and p27 (P = 0.04) in the dermis. Moreover, HER1 expression was significantly lower in the epidermis (P = 0.03) and significantly higher in the dermis (P = 0.0002).

ERK1 expression in the dermis is higher for lapatinib compared with erlotinib
When comparing low-molecular-weight HER inhibitors, a significantly higher expression of ERK1 in the dermis (P = 0.028) was detected for lapatinib compared with erlotinib (Fig. 2).

Variations in marker expression between HER inhibitors
Lower expression of pH1 (epidermis), CD68 (dermis), CD54 (in both layers), and CD4 (dermis) was observed for
samples from patients on cetuximab when compared with panitumumab ($P < 0.05$). A higher expression of CD8 was found in the dermis for panitumumab when compared with erlotinib, and a higher expression of CD1a (epidermis) was found for panitumumab and lapatinib compared with cetuximab ($P < 0.05$). Decreased expression of Ki67 was noted for cetuximab compared with lapatinib (epidermis; $P < 0.05$). STAT3 expression was decreased for cetuximab when compared with panitumumab in the epidermis and for cetuximab compared with all three other drugs in the dermis ($P < 0.05$).

**Atrophy, dyskeratosis, and dysmaturation in epidermis are less prominent with HER1/2i**

To determine differences in cutaneous architecture, archived histopathologic specimens from eight patients...
on each HERi were analyzed. Detailed histopathologic analyses are shown in Table 3 with representative histologic sections in Fig. 3.

The most frequent finding in the epidermis was atrophy, seen in 7 of 8 (87.5%) patients on cetuximab, 5 of 8 (62.5%) patients on erlotinib, 4 of 8 (50%) patients on panitumumab, and 2 of 8 (25%) patients on lapatinib. The extent of atrophy of lapatinib when compared with cetuximab, erlotinib, and panitumumab combined trended towards statistical significance ($P = 0.10$). Dyskeratosis and dysmaturation were more frequently observed with cetuximab and erlotinib (3 of 8 for both). Both of these findings were rare or absent with lapatinib (dyskeratosis, 0 of 8 patients; dysmaturation, 1 of 8 patients) and panitumumab (0 of 8 for both). Lapatinib was the only drug that was found to have monocytes within the epidermis (2 of 8, $P = 0.057$). Epidermal infiltrates were otherwise not frequent among all four drugs (0-2 of 8 patients).

Dermal presence of neutrophilic, monocytic, and eosinophilic infiltrates was more frequently seen with panitumumab (4 of 8), compared with cetuximab (2 of 8 patients) and erlotinib (2 of 8 patients). The presence of dermal monocytes was variable for all four drugs (1 of 8 for cetuximab, and 4 of 8 for erlotinib, lapatinib, and panitumumab).

Follicles were not found in the biopsies of two patients on cetuximab, and in one patient each on erlotinib, lapatinib, and panitumumab. Bacterial concretions were more frequently seen with erlotinib (5 of 7). The presence of a neutrophilic pustule was noted for all drugs, but in highest frequency with panitumumab (6 of 7). Follicular dyskeratosis was seen in 4 of 6 patients on cetuximab and 3 of 7 patients on erlotinib, as opposed to 1 of 7 for both lapatinib and panitumumab.

**Follicular neutrophilic infiltrates are more frequent during HER1i therapy**

Neutrophilic infiltrate within the follicle was less frequent for patients on lapatinib (3 of 7) as compared with cetuximab (5 of 6), erlotinib (3 of 7), and panitumumab (7 of 7), with a trend toward statistical significance ($P = 0.12$). Follicular monocytic (2 for each drug) and eosinophilic infiltrates (2 of 6 patients for cetuximab and 1 of 7 patients; all others) were similar among the four drugs.

Overall, alterations in eccrine glands were unusual, with erlotinib patients more likely to have changes (1 of 8 having dyskeratosis and 2 of 8 with necrosis). Similarly, sebaceous glands were evaluated for presence of an inflammatory infiltrate. Sebaceous gland infiltrates were most commonly found with panitumumab, where 3 of 5 specimens had infiltration present.

**Discussion**

Rash is the most frequently reported toxicity to HER1 inhibitors and, although not life threatening, often results in discontinuation and/or interruption of anticancer therapy, which may also affect clinical outcome. At present,
Altered keratinocyte growth and differentiation and enhanced inflammation induced by HER inhibition seem to play a critical role in the development of rash, especially in hair follicle epithelium (20). There is also some evidence that these agents may also alter the immune system (21). More recently, a preclinical model showed the role of tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) in the development of HER1i-associated skin rash and suggested a possible therapeutic role for anti-TNF agents (22). Several studies have aimed to identify histologic and immunohistochemical features of skin in patients undergoing therapy with HER1 inhibitors. In our study, we describe histologic and immunohistochemical differences in skin from patients treated with HER1 and HER1/2 inhibitors, consistent with clinical data of decreased rash severity after dual inhibition.

The limitations of this exploratory trial include small sample sizes in each of the four treatment groups. Even with these small sample sizes, significant differences were found between the epidermis and dermis in skin of patients treated with lapatinib, when compared with other HER inhibitors. A larger sample size would likely lead to more differences in biomarkers and histopathologic features and is currently being planned as part of a prospective study.

Use of the HER1/2 inhibitor lapatinib results in decreased HER1 phosphorylation and activation by reversibly binding to the cytoplasmic ATP-binding site, preventing subsequent downstream signaling of ERK-1/2 and PI3K/Akt (23). Clinical data suggest that the HER1/2i lapatinib is associated with a lower incidence of rash compared with single HER1 inhibitors (15–18). Consistent with these clinical data, our findings show a more intact immunohistochemical and histologic pattern in the skin of patients treated with the HER1/2 inhibitor lapatinib.

HER1 kinase activity is an important signal for pAKT/PI3K pathway activation (24), and pAKT has a key role in cell survival, with increasing activity during keratinocyte differentiation and stratification (25). The protective role of pAKT in skin treated with HER1 inhibitors has been shown in patients treated with erlotinib, in which greater pAKT expression at baseline correlated with decreased rash severity (26).

We also found decreased dermal expression of the proliferation marker K16 and the negative growth regulator...
p27 (27) in HER1/2i-treated patients, compared with those on HER1i therapy. In the skin of cetuximab-treated patients, p27 was upregulated in the epidermis (28), suggesting growth inhibition of basal keratinocytes. Lower p27 in HER1/2i-treated patients suggests decreased inhibition of proliferation in skin, consistent with lower skin toxicity. Increased ERK1 expression for lapatinib compared with erlotinib suggests greater pathway activity, which may account for lower severity of rash. Previous studies have shown that suppression of the HER1/ERK signaling pathway enhances skin inflammation by increasing chemokine expression in keratinocytes (10, 29).

Histologic findings in HER1i-induced rash include a mixed inflammatory infiltrate, suppurative folliculitis with follicular rupture, and epidermal dyskeratosis (28). Periocrine inflammation and dyskeratosis have also been reported. All of these findings have also been observed in our analyzed samples. These findings have also been shown in mice treated with an anti-EGFR monoclonal antibody, where follicular plugging with increased sebaceous gland size and a neutrophilic follicular infiltrate are observed (22). Enlargement of sebaceous units was not detected in our patients, which could potentially relate to timing of biopsies. The time from onset of rash to biopsy was not uniform in the current study. Early enlargement of sebaceous glands may be explained by a mouse study in which enlargement was noted to occur prior to the appearance of inflammatory infiltrates (22).

There was a lower incidence of epidermal atrophy in HER1/2i-treated patients. In addition, no patients on the HER1/2i lapatinib showed evidence of epidermal dyskeratosis, in contrast to 3 of 8 patients on the HER1i cetuximab and erlotinib. Similarly, follicular dyskeratosis was seen in 1 of 7 patients receiving the HER1/2i compared with 4 of 6 patients on cetuximab and 3 of 7 patients on erlotinib. In summary, a more benign histopathologic pattern was observed for patients on the HER1/2i lapatinib compared with the single inhibitors of HER1.

This phenomenon could be explained by the decreased expression of p27 for the HER1/2i as compared with the single HER1 inhibitors, which has been associated with impaired cell growth and differentiation in the follicle. This suggests improved keratinocyte survival, cell differentiation, and normalization of keratinization may possibly relate to an increase in pAKT activity as shown in our current immunohistochemistry data. Increased activity in the pAKT pathway may also lead to decreased dysmaturation and dyskeratosis in the epidermis with HER1/2 inhibition.

![Fig. 3. Histologic findings of representative specimens for each HER inhibitor. A, skin from a patient on lapatinib exhibiting a mild periadnexal and interstitial infiltrate consisting of mononuclear cells and neutrophils. B, specimen from a patient on cetuximab illustrating a more intense periadnexal neutrophilic infiltrate with mild follicular dyskeratosis. C, specimen from a patient on erlotinib showing an intense primarily neutrophilic perifollicular infiltrate with follicular dyskeratosis and mild epidermal atrophy. D, specimen from a patient on panitumumab showing a prominent follicular neutrophilic pustule with follicular dysmorphic features and dyskeratosis with overlying mild atrophy (magnification, ×10).](image-url)
Moreover, patients on cetuximab had lower levels of STAT3 as compared with the other drugs; as a differentiation marker this may lead to greater alterations in both epidermis and follicle and thus lead to such findings.

Inflammatory infiltrates for patients on lapatinib were predominantly composed of monocytes. Overall, there were few differences between all HER inhibitors when the inflammatory infiltrate was analyzed. However, fewer patients on lapatinib (3 of 7) had a neutrophilic follicular infiltrate as compared with cetuximab (5 of 6), erlotinib (5 of 7), and panitumumab (7 of 7), with a trend towards statistical significance \( (P = 0.12) \). This reduction in follicular inflammation with lapatinib is consistent with a lower incidence of rash (15). Similar numbers of monocytes and eosinophils within the follicle are identified for all four HER inhibitors. Moreover, dermal mononuclear infiltrates are less prominent for patients on cetuximab, which is consistent with lower levels of CD68, CD54, and CD4 seen in these patients.

Randomized clinical trials show a benefit of prophylactic management of HER1 inhibitor rash with minocycline (30) or a skin treatment regimen consisting of doxycycline, topical hydrocortisone, moisturizers, and sunscreen (31). These two published studies were conducted in patients receiving the anti-HER monoclonal antibodies cetuximab and panitumumab, respectively. There are no controlled studies on the management of rash from small molecule anti-HER agents erlotinib and lapatinib. However, our observations described here, showing a lower inhibition of the pAKT pathway and a decreased expression of K16 and p27 in the dermis, suggest that the design of trials against lapatinib-induced rash would require prophylactic interventions at lower doses or frequency, or conceivably that antirash interventions could be instituted in a reactive fashion, because the alterations at the cellular level seem to be of less significance, concordant with clinical observations showing that the lapatinib rash is of less frequency and severity as compared with erlotinib, lapatinib, or panitumumab. All these would necessitate confirmation in a separate study with the aforementioned HER inhibitory agents.

Taken as a whole, there are fewer histologic and immunohistochemical alterations in the skin of patients treated with an inhibitor of HER1/2 compared with single HER1 inhibitors. The finding of greater pAKT expression, decreased p27, and epidermal atrophy underscore cellular differences in rash induced by HER1/2i versus HER1i. These findings also suggest that interventions to identify risk factors, and prevent and treat rash due to HER1 and HER 1/2 inhibitors should be tailored to the causative agent.

**Disclosure of Potential Conflicts of Interest**

M.E. Lacouture: commercial research grant, GlaxoSmithKline, Bayer, and Hana; consultant/advisory board, Amgen, Bayer, Bristol-Meyers Squibb, Onyx, Threshold.

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**References**


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