UNIVERSITY OF CATANIA, SCHOOL OF MEDICINE

PHD PROGRAM IN PRECLINICAL AND CLINICAL PHARMACOLOGY

XXIII Cycle

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HISTOPATHOLOGIC AND IMMUNOHISTOCHEMICAL CHARACTERIZATION OF RASH TO HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 1 (HER1) AND HER1/2 INHIBITORS IN CANCER PATIENTS

PhD Thesis

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Academic Year 2009/2010

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1. INTRODUCTION

Human epidermal receptor (HER) also known as EGFR, is a 170-kd transmembrane glycoprotein that belongs to the ErbB family of receptor tyrosine kinases. (1, 2). It consists of an extracellular ligand-binding domain, a hydrophobic transmembrane domain, and an intracellular domain that possesses tyrosine kinase activity. It is activated by EGF-like ligands, including EGF, transforming growth factor alpha, amphiregulin, heparin-binding EGF-like growth factor, betacellulin, and epiregulin. Overexpression of HER1, and alterations in its signaling networks, has detrimental effects on normal cell growth, resulting in tumorigenesis (2). HER normally plays an important role in the control of cell growth and differentiation (1). Human epidermal receptor inhibitors (HERi) have shown effectiveness against a wide variety of solid tumors. In particular, they are used in malignancies overexpressing HER1 and 2, including head and neck, breast, lung, pancreatic, and colorectal cancers (3, 4).

The rationale for the use of HER1i in anticancer therapy is their attenuation effect in HER signaling pathways that lead cell differentiation, proliferation, migration, angiogenesis, and apoptosis (5, 6).

1.1. HER TARGETED AGENTS

Two main classes of HER targeted agents for cancer therapy have been developed to date: extracellular and cytoplasmic. In particular, monoclonal antibodies (mAb) block the extracellular domain of the receptor, thereby preventing ligand-dependent activation and downstream signaling, while small molecule, Tirosyn Kinase inhibitors (TKI) that are orally administered, are low molecular weight compounds directed against the intracellular tyrosine kinase domain blocking the intracytoplasmic ATP-biding site on the receptor, thereby preventing downstream signal transduction (7).

Two anti-HER1 monoclonal antibodies, cetuximab and panitumumab, have been approved by the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMEA) to date. Cetuximab, a chimeric human-murine IgG1 monoclonal antibody that binds to the extracellular domain of HER, is approved for second-line treatment of metastatic colorectal carcinoma as well as for advanced squamous cell carcinoma of the head and neck (8). Panitumumab is a fully human IgG2 monoclonal antibody approved for treatment of metastatic colorectal carcinoma refractory to standard chemotherapy (9). A

number of other HER-binding monoclonal antibodies, including zalutumumab, nimotuzumab and matuzumab, are currently in clinical development.

Of the small molecule HER1 tyrosine kinase inhibitors, erlotinib is indicated as second-line therapy for advanced non-small cell lung cancer (NSCLC) and in combination with gemcitabine for advanced pancreatic cancer (10). Gefitinib was approved in 2003 for the treatment of locally advanced or metastatic NSCLC refractory to both platinum-based and docetaxel chemotherapies; however, in 2005 the FDA labeling was modified to only allow continued treatment of those patients who are benefiting or have benefited from gefitinib therapy (11).

Lapatinib ditosylate is an oral dual kinase inhibitor targeting both the HER1 and HER2 receptors. Increased expression and activation of HER1 and HER2 in breast cancer are associated with a high risk for recurrence after primary treatment and consequently a poor clinical outcome (12). HER1 is reportedly overexpressed in up to 30% of breast tumors and HER2 is reportedly overexpressed in up to 25% of the 1.5 million new breast cancers that are diagnosed annually worldwide (13). Lapatinib reversibly binds to the intracellular cytoplasmic ATP-binding site of the tyrosine kinase domain and

blocks receptor phosphorylation and activation, thereby blocking downstream signaling pathways, namely, simultaneous activation of extracellular signal–related kinase 1/2 and phosphatidylinositol 3 kinase/Akt (14) (Fig. 1) .

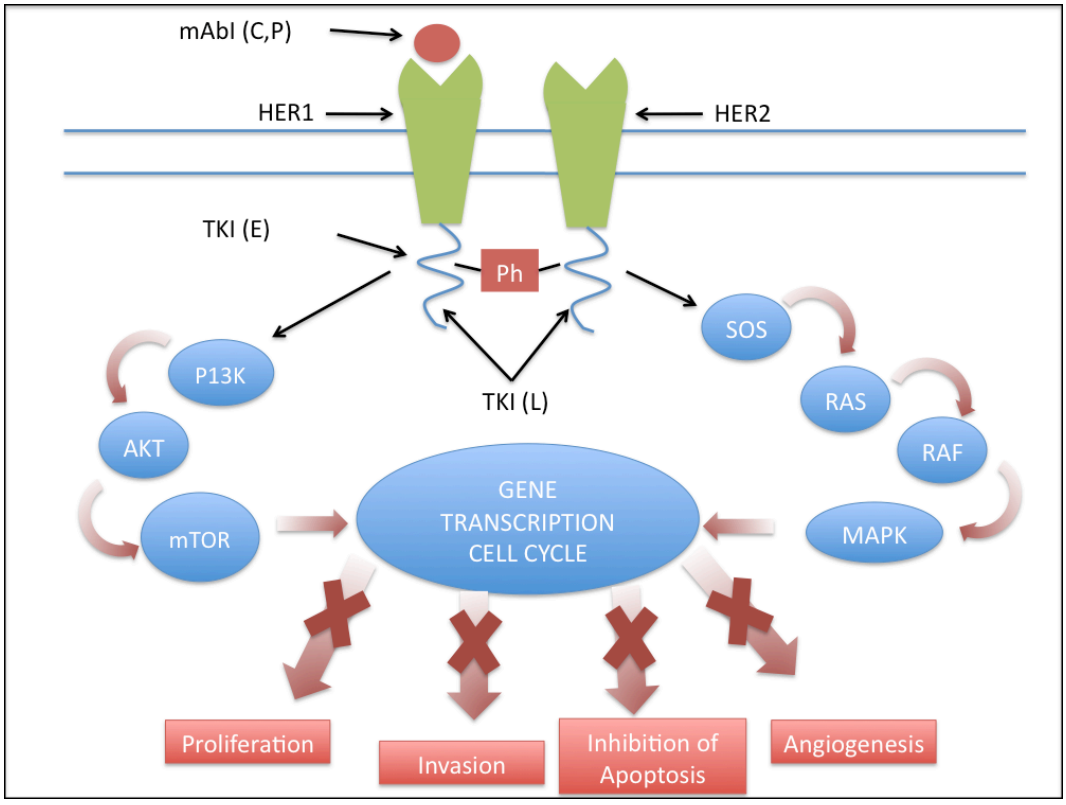

Figure 1. Mechanism of action of inhibitors of HER1 and HER 2.

mAbI= monoclonal antibody inhibitor; TKI=tyrosine kinase inhibitor; Ph= phosphorylation site; HER=human epidermal receptor; L= lapatinib; E= erlotinib; C=cetuximab; P= panitumumab. Monoclonal antibodies bind the extracellular domain, preventing ligand binding and subsequent activation of the receptor. Moreover, receptor internalization and degradation occurs after antibody binding. TKIs bind the intracytoplasmic domain of the receptors, preventing docking of the phosphate group, thereby abrogating activation and downstream signaling.

1.2. EFFECTS OF HER INHIBITORS ON SKIN

In skin, HER1 is primarily expressed in basal and suprabasl keratinocytes, sebocytes, and the outer root sheath of the hair follicle (15). Activation of HER1 by its ligands, epidermal growth factor (EGF), transforming growth factor-α (TGFα), amphiregulin, and heparin-binding EGF (HBEGF), has been shown to regulate keratinocyte proliferation, differentiation, migration and survival (16). HER-driven proliferation results in downstream activation of PI3K (phosphatidylinositol 3-kinase) -Akt and MAPK (mitogen activated protein kinase) pathways regulating keratinocyte survival, proliferation, and differentiation.

Blockade of HER1 in skin induces apoptosis in normal keratinocytes, which increases five-fold between therapy days 4 and 12, and which correlates with median time to rash onset in patients (17). 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 (18). HER1 inhibition induces early differentiation by upregulating the expression of terminal differentiation markers, such as KRT1 (keratin1) and KRT10 (19). In addition, increased STAT3 staining in the basal layer

of the epidermis (20) occurs, indicative of premature differentiation. Decreased expression of cytoskeletal proteins, f-actin-binding protein vinculin and the actin-binding protein ACTN1 (actinin-α1), resulting in decreased motility (21) ensues, along with increased attachment via cadherin-associated protein CTNND2 (catenin-δ2) and DSG2 (desmoglein 2) (22).

1.3. PAPULOPUSTULAR RASH DUE TO HER INHIBITORS

Although HERi are usually well-tolerated compared to standard cancer therapies such as chemotherapy and radiation, they may be associated with significant side effects. Cutaneous reactions are the most common adverse effects, and are associated with all HER inhibitors. Consequently, dermatologic toxicities are considered to be a class-specific side effects that are likely due to HER inhibition. Cutaneous and mucous membrane toxicities most commonly associated with HERi include papulopustular rash, paronychia, hair changes, dry skin, hypersensitivity reacton and mucositis (23).

Other common side effects include diarrhea, nausea, vomiting and asthenia.

The most common clinical toxicity associated with the use of HERi is a papulopustular (sometimes referred to as acneiform) rash that develops in up to 90% of patients (24-26).

This rash is clinical characterized by erythematous papulopustules in 45-100% of patients and commonly affects the face and upper body (back and chest) (Fig.2) while scalp, extremities, abdomen and buttocks are affected less frequently (27).

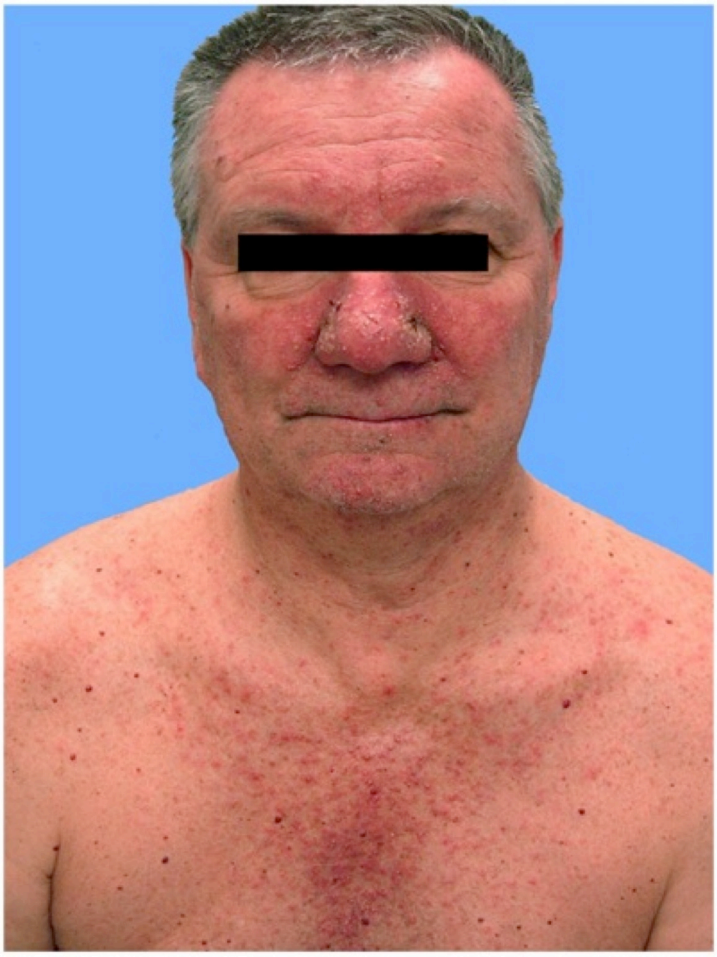

Figure 2. Clinical presentation of papulopustular rash due to HER inhibitors.

HER-induced skin rash typically occurs within 8-10 days with a peak at 2 weeks of treatment. The most commonly involved sites are face, upper chest and back. Permanent scarring generally does not occur; however, significant scarring and postinflammatory hyperpigmentation following resolution of severe rash have been described (23, 28). Papulopustular eruptions seem to be dose-

dependent (29).

Histologic analysis of HERi-induced skin rash is characterized by a suppurative folliculitis. An early finding may consist of T lymphocyte infiltration surrounding the follicular infindibulum. Consequently, a predominantly neutrophilic infiltrate is seen that may also include lymphocytes and histiocytes, that surround follicles. Severe eruptions may reveal destruction of follicles.

In contrast to the findings in acne vulgaris, sebaceous glands are not affected in HERi-induced papulopustular rash. Thinning of the stratum corneum and a loss of the normal basketweave appearance is observed. Enlarged follicles may contain keratin plugs (28, 30-32).

The mechanism by which HER1i are associated with cutaneous toxicity is unknown, but the alteration of physiologic HER1-mediated signaling processes in the epidermis and hair follicle seems to play a key role. It has been shown that HERi leads to a decrease in expression of Ki-67 within the epidermis, thereby reflecting inhibition of keratinocyte proliferation, as well as leading to induction of p27, a cyclin-dependent kinase inhibitor that arrests the growth of keratinocytes (30, 33).

Papulopustular rash due to HER1i has a negative impact as it relates to quality of life (34), cost (35), secondary infections (36) and

ability to mantain antineoplastic therapy without interruption (37), all of which may also affect clinical outcome.

2. EXPERIMENTAL DESIGN

Clinically, HER1/2 inhibition by lapatinib results in a lower incidence of rash (41%) (38), when compared to the HER1 inhibitors erlotinib, cetuximab and panitumumab 75-90% (24-26). Based on rash severity, patients on HER1/2i are less likely to require dose modifications when compared to 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 histological and immunohistochemical characteristics that may explain some of the clinical differences in skin toxicity between HER1 inhibitors (HER1i) and the HER1/2 inhibitors (HER1/2i).

3. MATERIALS AND METHODS

PATIENTS

Upon IRB approval, existing medical records including archived skin biopsy specimens, 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 (IHC) studies were performed on 5 μ m sections of formalin-fixed paraffin-embedded tissue by using an Envision kit (Dako, Carpinteria, CA), 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, San Juan Capistrano, CA) 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 another 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 (39). Seventeen IHC biomarkers were used for each case and included Keratin 1 (KRT1), HLA-DR, Extracellular Regulated Kinase 1 (ERK1), K16, Ki67, p27, pAKT, HER1, pHER1, CD68, CD54, CD20, CD11b, CD4, CD8, CD1a, and STAT3 (Table 1).

Antigen	Function	Manufacturer PCF	Dilution PCF
KRT1	Terminal differentiation marker	Sigma	1:200
HLA-DR	MHC II (inflammatory infiltrate subtype)	Santa Cruz	1:200
ERK1	MAPK pathway, has a role in HER1-driven control of proliferation and inflammatory response	Cell Signaling	1:25
K16	Hyperproliferation Marker	Thermo	1:200
Ki67	Proliferative marker	Dako	1:200
p27	Negative growth regulator	Dako	1:200
pAKT	Signal transduction marker	Lab Vision	1:100
HER1	Human Epidermal Receptor 1	Dako	1:100
CD68	Macrophage (inflammatory infiltrate subtype)	Dako	1:100
CD54	Endothelium/macrophages/Lymphocytes (inflammatory infiltrate subtype)	Santa Cruz	1:200
CD20	B cells (inflammatory infiltrate subtype)	Dako	1:5000
CD11b	Leucocytes (Monocytes, Granulocytes, Macrophage, NK) (inflammatory infiltrate subtype)	Abcam	1:200
CD8	T Cells (inflammatory infiltrate subtype)	Dako	1:200
CD4	T Cells (inflammatory infiltrate subtype)	Lab Vision	1:200
CD1a	MHC I (inflammatory infiltrate subtype)	Dako	1:200
STAT3	Differentiation marker	Neomarkers	1:200
pHER1	Phosphorylated Human Epidermal 1 Receptor	Zymed	1:400

Table 1. Immunohistochemical markers analyzed. KRT1=Keratin 1; ERK=, Extracellular Regulated Kinase 1; HER1=Human Epidermal Receptor 1; pHER1=Phosphorylated Human Epidermal Receptor 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 hematoxylin and eosin was performed and specimens were submitted to two dermatopathologists for independent blinded assessment of the epidermis, dermis, follicle and inflammatory infiltrate (P.G. and J.G). Biopsies were separately evaluated for the presence of epidermal, dermal, follicular, eccrine gland, and sebaceous gland alterations. Specifically, the epidermis was evaluated for 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 IHC 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 analysis of variance methods. For each specimen positive staining calculations were determined. A two-factor nested repeated measures analysis of variance was used, with drug as the between subject factor, with subject nested within drug and skin layer (epidermis or dermis) as the within subject factor. A drug by skin layer interaction term is 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 analysis of variance (ANOVA) was performed 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 less than 0.05 were considered statistically significant.

4. 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%), grade 3 (n=2, 25%); for cetuximab, severity was grade 1 (n=4, 50%), grade 2 (n=3, 37.5%), grade 3 (n=1, 12.5%); for panitumumab, 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%).

Medication	Number of Patients	Age Mean (Range)	Gender F:M	Cancer Type	Rash Severity (at the time of biopsy)
Cetuximab	8	54.5 (34-72)	4:4	2 mCRC 4 CRC 1 Gastric cancer 1 HNSCC	4 Grade I 3 Grade II 1 Grade III
Erlotinib	8	66.7 (46-81)	2:6	7 Lung cancer 1 mSCC	5 Grade I 1 Grade II 2 Grade III
Lapatinib	8	55.7 (17-62)	7:1	7 Breast cancer 1 Medulloblastoma	3 Grade I 4 Grade II 1 Grade III
Panitumamab	8	65 (39-81)	5:3	4 CRC 4 mCRC	2 Grade I 3 Grade II 3 Grade III

Table 2. Patient characteristics (N=32). mCRC=metastatic colorectal cancer; HNSCC= Head and neck squamous cell carcinoma; mSCC= metastatic squamous cell carcinoma.

DUAL HER1/2 INHIBITION IS ASSOCIATED WITH INCREASED PAKT, DECREASED K16 AND P27 IN SKIN

Significant IHC results are summarized in Figure 3. When comparing HER1/2i to the 3 HER1i, IHC analysis revealed a significantly increased expression of pAKT in both epidermis (p=0.043) and dermis (p=0.007) (Fig. 4), and a decreased expression of K16 (p=0.032) and p27 (p=0.04) in the dermis. Also, HER1 expression was significantly lower in the epidermis (p=0.03) and significantly higher in the dermis (p=0.0002).

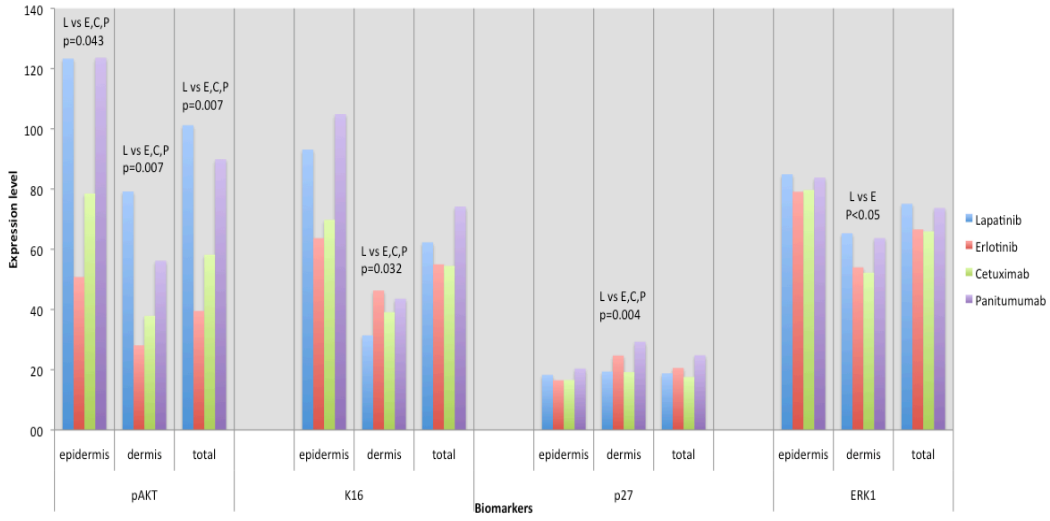

Figure 3. Most notable immunohistochemical results.

Immunohistochemical analysis highlighting significant differences in epidermis and dermis between different agents. L= lapatinib; E= erlotinib; C=cetuximab; P=panitumumab; pAkt= phospho Akt; K16=keratin 16; ERK1= Extracellular Regulated Kinase 1

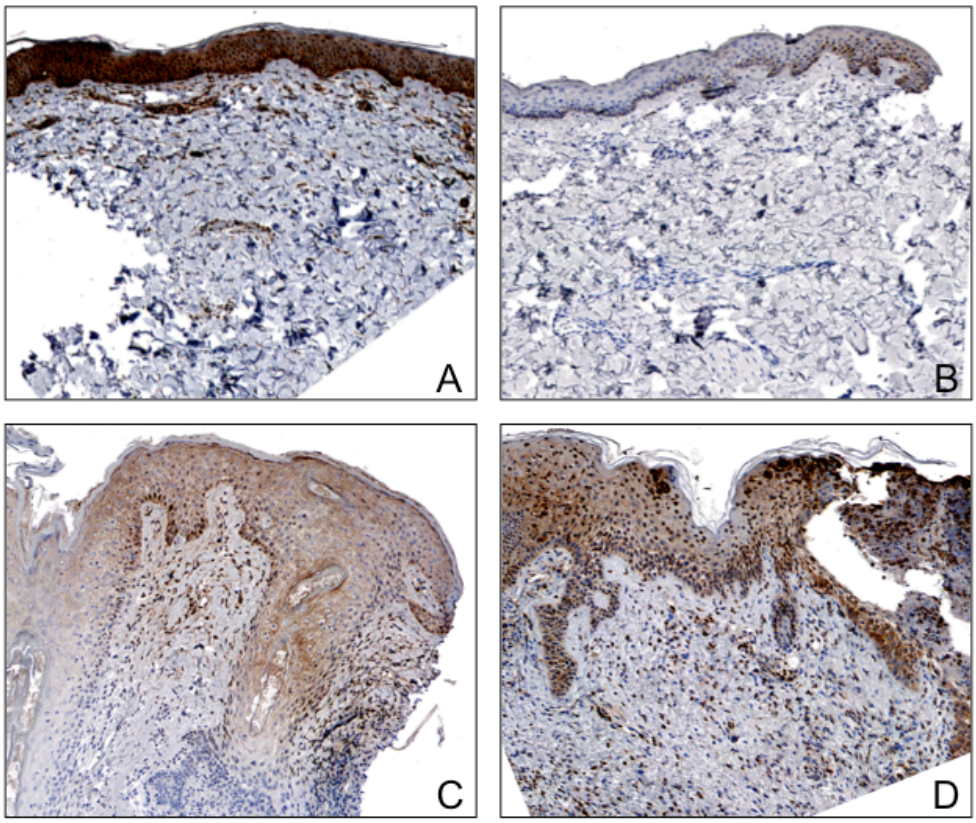

Figure 4. Immunohistochemistry of representative specimens for each HER inhibitor for pAKT.

A) Specimen from a patient on lapatinib showing increased pAKT expression in the epidermis as compared to cetuximab (B), erlotinib (C), and panitumumab (D), (X40 magnification).

ERK1 EXPRESSION IN THE DERMIS IS HIGHER FOR LAPATINIB COMPARED TO 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 to erlotinib.

VARIATIONS IN MARKER EXPRESSION BETWEEN HER INHIBITORS

Lower expression of pHER1 (epidermis), CD68 (dermis), CD54 (in both layers), and CD4 (dermis) was observed for samples from patients on cetuximab when compared to panitumumab ($p<0.05$). A higher expression of CD8 was found in the dermis for panitumumab when compared to erlotinib and a higher expression of CD1a (epidermis) was found for panitumumab and lapatinib compared to cetuximab ($p<0.05$). Decreased expression of Ki67 was noted for cetuximab compared to lapatinib (epidermis) ($p<0.05$). STAT3 expression was decreased for cetuximab when compared to panitumumab in the epidermis and for cetuximab compared to 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 8 patients on each HERi were analyzed. Detailed histopathologic analyses are shown in Table 4 with representative histologic sections in Figure 5.

	Total Number of Cases					
Histopathologic Finding	**Cetuximab**	**Erlotinib**	**Lapatinib**	**Panitumumab**	**[1]pvalue**	**[2]pvalue**
Ulceration	1	1	**0**	2	0.89	0.55

Parakeratosis	1	2	1	**0**	0.89	0.99
Acanthosis	1	1	0	0	0.99	0.99
Epidermal Atrophy	7	5	**2**	4	0.10	0.10
Epidermal Dysmaturation	2	3	1	**0**	0.44	0.99
Epidermal Dyskeratosis	3	3	**0**	**0**	0.053	0.30
Epidermal Neutrophilc Infiltrate	1	1	**0**	1	0.99	0.55
Epidermal Monocytic Infiltrate	0	0	2	0	0.23	0.057
Epidermal Eosinophilic Infiltrate	0	0	0	1	0.99	0.99
Dermal Neutrophilic Infiltrate	2	0	2	4	0.18	0.99
Dermal Monocytic Infiltrate	**1**	4	4	4	0.32	0.68
Dermal Eosinophilic Infiltrate	2	2	0	0	0.29	0.55
Follicular concretions	3	5	**2**	3	0.59	0.42
Follicular Neutrophilic Pustule	**3**	4	**3**	6	0.56	0.69
Dysmorphic follicle	4	2	2	4	0.63	0.68
Follicular dyskeratosis	4	3	**1**	**1**	0.34	0.39
Follicular Neurtrophilic infiltrate	5	5	**3**	7	0.28	0.12
Follicular Monocytic Infiltrate	2	2	2	2	0.99	0.99
Follicular Eosinophilic Infiltrate	2	1	1	1	0.99	0.99
Eccrine Dyskeratosis	0	1	0	0	0.99	0.99
Eccrine Necrosis	0	2	0	1	0.59	0.55
Eccrine Infiltrate	0	0	0	0	N/A	N/A
Sebaceous Infiltrate	0	1	1	3	0.34	0.99

Table 3. Histopathologic results. [1] p-value among the four drugs; [2] p-value between lapatinib compared to all 3 drugs. Bolded values are representative of the drug with the lowest number of specimens with that particular histologic finding.

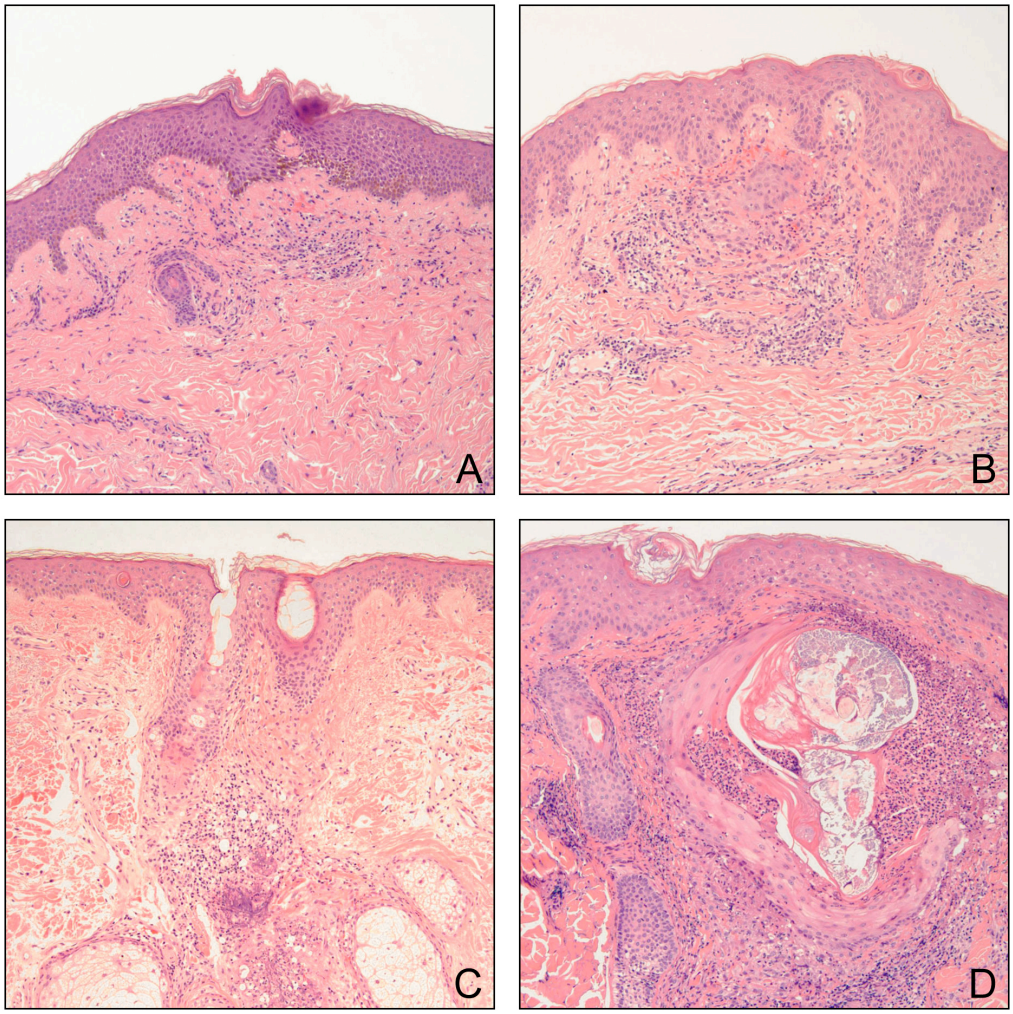

Figure 5. Histologic findings of representative specimens for each HER inhibitor.

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 demonstrating a prominent follicular neutrophilic pustule with follicular dysmorphic features and dyskeratosis with overlying mild atrophy (X10 magnification).

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. A comparison of the extent of atrophy between lapatinib compared to 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 amongst all four drugs (0-2 of 8 patients).

Dermal presence of neutrophilic, monocytic, and eosinophilic infiltrates were more frequently seen with panitumumab (4 of 8), compared to cetuximab (2 of 8 patients) and erlotinib (2 of 8 patients). Presence of dermal monocytes were 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 2 patients on cetuximab, and in 1 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 to cetuximab (5 of 6), erlotinib (5 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 amongst 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.

5. DISCUSSION AND CONCLUSIONS

Skin 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, the exact mechanism of this rash is not clearly understood. Altered keratinocyte growth and differentiation and enhanced inflammation induced by HER inhibition seems to play a critical role in the development of rash, especially in hair follicle epithelium (40). There is also some evidence that these agents may also alter the immune system (41). More recently, a preclinical model demonstrated 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 (32). 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 to other HER inhibitors. A larger sample size would likely lead to more differences in biomarkers and histopathological 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 (12). Clinical data suggest that the HER1/2i lapatinib is associated with a lower incidence of rash compared to single HER1 inhibitors (24-26, 38). Consistent with these clinical data, our findings show a more intact immunohistochemical and histological 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 (42), and pAKT has a key role in cell survival, with increasing activity during keratinocyte differentiation and stratification (43) 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 (44).

We also found decreased dermal expression of the proliferation marker K16 and the negative growth regulator p27 (33) in HER1/2i treated patients, compared to those on HER1i therapy. In the skin of cetuximab treated patients, p27 was upregulated in epidermis (30), 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 to erlotinib, suggests greater pathway activity, which may account for lower inflammmation of rash. Previous studies have demonstrated that suppression of the HER1/ERK signaling pathway enhances skin inflammation by increasing chemokine expression in keratinocytes (18, 45).

Histologic findings in HER1i-induced rash include a mixed inflammatory infiltrate, suppurative folliculitis with follicular rupture, and epidermal dyskeratosis (30). Perieccrine inflammation and dyskeratosis have also been reported. All of these findings have also been observed in our analyzed samples. These findings have also been demonstrated in mice treated with an anti-EGFR monoclonal antibody, where follicular plugging with increased sebaceous gland size and a neutrophilic follicular infiltrate are observed (32). 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 (32).

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 to 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 to the single inhibitors of HER1.

This phenomenon could be explained by the decreased expression of p27 for the HER1/2i as compared to 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 demonstrated in our current IHC data. Increased activity in the pAKT pathway may also

lead to decreased dysmaturation and dyskeratosis in the epidermis with HER1/2 inhibition. Moreover, patients on cetuximab had lower levels of Stat3 as compared to 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 HERi when was analyzed the inflammatory infiltrate. However, fewer patients on lapatinib (3 of 7) had a neutrophilic follicular infiltrate as compared to 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 (38). Similar numbers of monocytes and eosinophils within the follicle are identified for all four HERi. Moreover, dermal monocytic 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 (46) or a skin treatment regimen consisting of doxycycline, topical hydrocortisone,

moisturizers, and sunscreen (47). Both of these 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 to small molecule anti-HER agents erlotinib and lapatinib. However, our observations described here in 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 anti-rash interventions could be instituted in a reactive fashion, since the alterations at the cellular level appear to be of less significance, concordant with clinical observations showing that the lapatinib rash is of less frequency and severity as compared to erlotinib, cetuximab, or panitumumab. All of which 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 to single HER1 inhibitors. The finding of greater pAKT expression, decreased p27 and epidermal atrophy underscore cellular differences in skin toxicity induced by HER1/2i vs HER1i. These findings also suggest that interventions to

identify risk factors, prevent, and treat rash due to HER1 and HER 1/2 inhibitors should be tailored to the causative agent.

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ACKNOWLEDGEMENTS

I would like to express my sincere thanks to my advisor Prof. Dennis West, for his invaluable support, guidance and patience throughout this project and these 2 years in the US.

I also wish to thank Prof. Mario Lacouture, for his continuous assistance, advice, support and valuable contribution to this project and the sharing of his expertise and scientific knowledge.

I am grateful to Prof. Amy Paller, Chair of the Department of Dermatology, Northwestern University for her support and the opportunity to conduct research in a very conducive environment.

I would like to thank Prof. Mary Martini, Director Pigmented Lesion Clinic, for her continuous personal and scientific support throughout my time in Chicago.

Special thanks to all Department of Dermatology, Northwestern University and Clinical staff, and especially the Clinical Trials Unit staff, for their wonderful assistance during the 2 years.

I would also like to express my gratitude to Prof. Giuseppe Micali, my mentor, for his invaluable contribution throughout my scientific growth and for his continuous encouragement in realizing my PhD research experience.

I am also very grateful to Prof. Renato Bernardini, for his continuous and valuable support in making this experience possible.

Part of this thesis has been accepted for publication:

*Nardone B, Nicholson K, Newman M, Guitart J, Gerami P, Talarico N, Yang XJ, Rademaker A, West DP, Lacouture ME**. Histopathologic and immunohistochemical characterization of rash to human epidermal growth factor receptor 1 (HER1) and HER1/2 inhibitors in cancer patients. *Clin Cancer Res 2010; Sep 1;16(17):4452-60.*

*Special thanks go to all of the authors for their valuable contribution to this project.

APPENDIX A

IMMUNOHISTOCHEMICAL DATA FROM ACIS II (CHROMAVISION MEDICAL SYSTEMS, INC.)

KRT1

Dearm Path Number	Area	Intensity	Percentage (%)
DP07-2010	Epidermis	150,00	64,22
	Dermis	111,75	5,80
DP07-3486	Epidermis	174,75	82,46
	Dermis	112,50	3,82
DP07-2495	Epidermis	168,25	70,56
	Dermis	101,50	3,53
DP07-1561	Epidermis	178,25	82,88
	Dermis	102,50	2,85
DP07-5876	Epidermis	142,25	64,74
	Dermis	114,50	4,07
DP07-5878	Epidermis	165,75	87,12
	Dermis	109,25	3,70
DP08-541	Epidermis	168,00	95,20
	Dermis	123,50	6,98
DP08-2678	Epidermis	146,50	86,38
	Dermis	105,25	2,86
DP07 10082-A	Epidermis	160,00	76,04
	Dermis	109,25	3,60
DP07 17053	Epidermis	147,00	87,73
	Dermis	100,00	2,56
DP07 17250	Epidermis	179,50	82,87
	Dermis	113,00	2,98
DP06-2974	Epidermis	153,50	76,63
	Dermis	98,75	5,28
DP07 19209	Epidermis	136,75	80,79
	Dermis	103,25	3,88
DP07-6221	Epidermis	203,25	92,96

	Dermis	103,50	5,48
DP07-8218	Epidermis	126,50	87,99
	Dermis	97,25	3,44
DP2619-06	Epidermis	133,00	82,72
	Dermis	100,50	3,30
DP845-07	Epidermis	171,75	90,49
	Dermis	101,25	4,83
DP07-7169	Epidermis	159,75	87,53
	Dermis	106,50	1,86
DP07-8617	Epidermis	131,50	79,55
	Dermis	108,00	3,44
DP254-07	Epidermis	140,75	81,01
	Dermis	110,00	4,87
DP07-6294	Epidermis	155,75	69,39
	Dermis	116,75	4,53
DP07-8786	Epidermis	167,75	74,54
	Dermis	107,25	2,78
DP06-11523	Epidermis	172,00	83,57
	Dermis	103,50	6,97
DP07-6295	Epidermis	127,25	89,53
	Dermis	94,75	2,30
DP07-1826	Epidermis	147,25	85,68
	Dermis	92,50	3,27
DP07-5446	Epidermis	157,50	95,58
	Dermis	114,25	6,83
DP07-4746	Epidermis	122,25	79,15
	Dermis	99,50	3,54
DP08 15669	Epidermis	157,25	95,74
	Dermis	97,50	4,90
DP07 18707	Epidermis	134,25	88,83
	Dermis	94,75	4,92
DP07-1157	Epidermis	137,25	84,06
	Dermis	101,00	4,29
DP07-1905	Epidermis	135,50	60,86
	Dermis	105,75	6,21
DP06-2953	Epidermis	152,75	74,93

	Dermis	114,75	4,37

HLA-DR

Derm Path Number	Area	Intensity	Percentage (%)
DP07-2010	Epidemis	166,50	6,23
	Dermis	166,00	24,95
DP07-3786	Epidemis	167,00	7,51
	Dermis	160,50	13,02
DP07-2495	Epidemis	163,00	8,17
	Dermis	160,50	13,71
DP07-1561	Epidemis	160,50	7,67
	Dermis	159,00	18,55
DP07-5876	Epidemis	156,00	6,75
	Dermis	162,00	13,92
DP07-5878	Epidemis	165,00	7,60
	Dermis	159,00	12,57
DP08-541	Epidemis	159,00	15,24
	Dermis	152,50	11,33
DP08-2678	Epidemis	165,00	9,85
	Dermis	171,50	37,03
DP07 10082-A	Epidemis	165,50	11,15
	Dermis	167,00	18,84
DP07 17053	Epidemis	160,50	7,61
	Dermis	149,00	7,55
DP07-17250	Epidemis	172,00	10,33
	Dermis	158,50	10,40
DP06 2974	Epidemis	134,00	17,15
	Dermis	133,00	16,41
DP07 19209	Epidemis	137,00	6,10
	Dermis	133,00	14,77
DP07-6221	Epidemis	140,00	13,91
	Dermis	123,00	16,90
DP07-8218	Epidemis	138,50	8,50
	Dermis	136,00	36,75
DP2619-06	Epidemis	146,50	13,17
	Dermis	150,50	48,61

DP854-07	Epidemis	138,50	28,51
	Dermis	134,50	51,31
DP07-7619	Epidemis	129,50	21,29
	Dermis	128,00	20,54
DP07-8617	Epidemis	138,50	29,82
	Dermis	133,50	46,86
DP254-07	Epidemis	143,00	13,53
	Dermis	149,00	64,56
DP07-6294	Epidemis	128,50	14,88
	Dermis	128,50	16,92
DP07-8786	Epidemis	143,00	18,28
	Dermis	135,00	25,72
DP07-6295	Epidemis	154,50	2,23
	Dermis	158,50	9,44
DP06-11523	Epidemis	128,00	16,31
	Dermis	122,50	23,10
DP07-1826	Epidemis	163,50	5,24
	Dermis	154,00	5,80
DP07-5446	Epidemis	170,50	10,26
	Dermis	177,00	19,06
DP07-4746	Epidemis	166,00	11,66
	Dermis	174,00	19,08
DP08 15667	Epidemis	157,50	1,69
	Dermis	148,00	6,06
DP07 18707	Epidemis	169,00	4,54
	Dermis	156,00	7,04
DP07-1157	Epidemis	157,00	3,51
	Dermis	153,00	7,09
DP07-1925	Epidemis	133,50	1,01
	Dermis	139,00	6,54

ERK1

Derm Path Number	Area	Indensity	Percentage (%)
DP07-2495	Epidermis	111,67	72,58
	Dermis	119,40	47,16
DP07-1561	Epidermis	133,00	84,04

	Dermis	137,20	56,13
DP07 17250	Epidermis	130,33	90,26
	Dermis	129,40	48,92
DP07 17053	Epidermis	128,00	41,68
	Dermis	130,00	47,78
DP07 10082-A	Epidermis	115,67	94,05
	Dermis	138,80	54,00
DP07-8786	Epidermis	121,00	61,65
	Dermis	118,40	38,68
DP07-2010	Epidermis	136,33	97,27
	Dermis	144,60	53,27
DP07-5876	Epidermis	113,00	38,83
	Dermis	114,00	41,06
DP07-5878	Epidermis	121,33	91,09
	Dermis	127,20	56,98
DP07-6294	Epidermis	127,00	94,33
	Dermis	131,60	51,45
DP08-541	Epidermis	148,67	85,98
	Dermis	135,80	47,76
DP08-2678	Epidermis	111,67	54,24
	Dermis	125,80	50,92
DP07-3486	Epidermis	119,00	92,31
	Dermis	143,60	59,70
DP07-6221	Epidermis	99,67	36,45
	Dermis	103,00	38,90
DP07-8218	Epidermis	111,67	75,04
	Dermis	123,40	50,28
DP07 19209	Epidermis	108,33	58,81
	Dermis	132,40	52,66
DP06-2953	Epidermis	145,33	88,16
	Dermis	139,60	50,28
DP06-2974	Epidermis	134,33	73,70
	Dermis	125,60	46,66
DP06-11523	Epidermis	146,00	42,35
	Dermis	120,20	20,50
DP254-07	Epidermis	108,00	88,97

	Dermis	122,80	51,01
DP845-07	Epidermis	120,00	69,73
	Dermis	120,40	51,62
DP07-6295	Epidermis	132,00	34,77
	Dermis	133,40	49,93
DP07-7169	Epidermis	113,67	65,57
	Dermis	121,80	49,72
DP07-8617	Epidermis	112,67	91,47
	Dermis	127,40	49,15
DP07-1826	Epidermis	98,33	27,95
	Dermis	110,60	28,31
DP07-5446	Epidermis	161,00	39,49
	Dermis	136,80	42,94
DP07-4746	Epidermis	126,00	72,15
	Dermis	130,80	46,69
DP08 15669	Epidermis	122,67	79,07
	Dermis	129,60	38,02
DP07-1905	Epidermis	114,33	25,28
	Dermis	117,00	34,58
DP07-1157	Epidermis	100,67	50,57
	Dermis	124,40	28,48
DP07-18707	Epidermis	118,33	45,03
	Dermis	113,80	48,07

K16

Derm Path Number	**Area of Interest**	**Intensity**	**Percentage (%)**
DP06-2953	Epidermis	174,00	88,17
	Dermis	132,00	13,22
DP06-2974	Epidermis	114,50	65,78
	Dermis	118,00	43,77
DP2619-06	Epidermis	117,00	15,80
	Dermis	120,00	44,43
DP07-1561	Epidermis	147,50	88,73
	Dermis	116,00	38,96
DP07-2010	Epidermis	163,00	87,87

	Dermis	138,50	26,62
DP07-2495	Epidermis	120,00	69,28
	Dermis	127,00	35,55
DP07-3486	Epidermis	152,50	93,27
	Dermis	124,50	23,11
DP07-5876	Epidermis	132,50	32,94
	Dermis	120,50	19,80
DP07-5878	Epidermis	156,50	51,97
	Dermis	145,50	19,28
DP07-6221	Epidermis	123,50	30,74
	Dermis	127,00	24,98
DP07-6294	Epidermis	127,50	74,02
	Dermis	119,00	27,21
DP07-7169	Epidermis	115,50	60,75
	Dermis	113,50	24,40
DP07-8218	Epidermis	117,50	66,21
	Dermis	110,00	34,11
DP07-8617	Epidermis	114,50	44,35
	Dermis	114,50	41,97
DP07-8786	Epidermis	106,50	31,62
	Dermis	109,00	19,42
DP07 10082-A	Epidermis	126,00	77,53
	Dermis	138,00	18,71
DP07-17053	Epidermis	131,50	58,85
	Dermis	130,00	15,04
DP07 17250	Epidermis	124,50	72,94
	Dermis	130,00	15,78
DP07 19209	Epidermis	120,00	56,88
	Dermis	122,00	28,31
DP254-07	Epidermis	117,50	23,31
	Dermis	122,00	43,02
DP845-07	Epidermis	103,50	23,11
	Dermis	100,00	13,10
DP08-541	Epidermis	143,50	79,14
	Dermis	137,00	20,84
DP08-2678	Epidermis	149,00	82,64

	Dermis	140,00	27,08
DP06-11523	Epidermis	128,50	86,60
	Dermis	120,50	64,95
DP07-1157	Epidermis	131,00	91,98
	Dermis	112,00	59,14
DP07-1826	Epidermis	123,00	85,11
	Dermis	117,50	52,24
DP07-1905	Epidermis	111,50	24,02
	Dermis	121,00	33,22
DP07-4746	Epidermis	135,00	88,96
	Dermis	101,00	61,93
DP075446	Epidermis	122,00	30,94
	Dermis	110,00	44,50
DP07-6295	Epidermis	141,50	89,51
	Dermis	114,00	45,91
DP07-18707	Epidermis	111,50	28,20
	Dermis	112,00	36,72
DP08-15669	Epidermis	121,50	94,45
	Dermis	124,50	57,08

p27

Derm Path Number	Area	Intensity	Percentage (%)
DP07-2010	Epidermis	166,40	20,31
	Dermis	144,50	29,59
DP07-3486	Epidermis	154,60	7,83
	Dermis	144,25	31,69
DP07-2495	Epidermis	155,40	11,09
	Dermis	157,50	30,89
DP07-1561	Epidermis	154,80	8,89
	Dermis	126,75	8,99
DP07-5876	Epidermis	138,20	5,24
	Dermis	124,25	12,96
DP07-5878	Epidermis	144,00	5,50
	Dermis	138,25	17,95
DP08-541	Epidermis	141,20	29,51
	Dermis	131,25	12,86

DP08-2678	Epidermis	143,00	7,06
	Dermis	128,75	15,38
DP07 10082-A	Epidermis	135,20	14,27
	Dermis	122,00	15,60
DP07 17053	Epidermis	152,00	6,60
	Dermis	141,75	18,01
DP07 17250	Epidermis	147,20	22,76
	Dermis	131,25	12,28
DP06-2974	Epidermis	135,40	10,71
	Dermis	117,50	14,35
DP07-6221	Epidermis	133,00	10,87
	Dermis	125,75	17,83
DP07-7169	Epidermis	110,60	8,96
	Dermis	122,75	19,36
DP07-8218	Epidermis	135,60	7,05
	Dermis	127,75	15,37
DP07 19209	Epidermis	130,80	7,04
	Dermis	125,00	15,54
DP845-07	Epidermis	140,80	29,09
	Dermis	128,50	25,17
DP07-6294	Epidermis	136,60	6,42
	Dermis	121,75	13,99
DP07-8786	Epidermis	147,20	10,61
	Dermis	124,25	16,98
DP06-11523	Epidermis	150,40	23,95
	Dermis	122,25	20,99
DP07-6295	Epidermis	127,00	13,84
	Dermis	115,00	19,88
DP07-8617	Epidermis	140,80	6,70
	Dermis	125,75	19,44
DP07-1826	Epidermis	125,40	22,62
	Dermis	111,50	19,91
DP254-07	Epidermis	130,60	11,83
	Dermis	121,25	20,01
DP07-5446	Epidermis	145,80	23,01
	Dermis	123,75	17,49

DP07-4746	Epidermis	129,20	10,93
	Dermis	120,75	13,69
DP07 15669	Epidermis	125,60	8,62
	Dermis	122,75	31,01
DP2619-06	Epidermis	135,00	6,13
	Dermis	122,00	11,37
DP07-1905	Epidermis	145,20	9,08
	Dermis	131,75	17,63
DP07-1157	Epidermis	145,60	11,97
	Dermis	129,00	11,17
DP07-18707	Epidermis	136,80	14,47
	Dermis	127,25	11,59

pAKT

Derm Path Number	**Area**	**Intensity**	**Percentage(%)**
DP07-2010	Epidermis	145,40	94,09
	Dermis	115,50	21,94
DP07-2495	Epidermis	166,80	97,75
	Dermis	117,50	14,36
DP07-3486	Epidermis	154,00	95,74
	Dermis	139,50	62,99
DP07-1561	Epidermis	135,80	96,69
	Dermis	131,50	59,65
DP07-5876	Epidermis	127,60	52,84
	Dermis	142,50	69,46
DP07-5878	Epidermis	161,60	99,01
	Dermis	149,00	80,81
DP08-541	Epidermis	174,00	96,43
	Dermis	154,00	58,12
DP08-2678	Epidermis	133,20	92,76
	Dermis	143,50	72,31
DP08 10082-A	Epidermis	150,00	94,87
	Dermis	139,50	37,22
DP07-17053	Epidermis	155,20	81,65
	Dermis	151,50	29,05
DP07 17250	Epidermis	167,20	97,19

	Dermis	132,00	41,07
DP06-2794	Epidermis	146,40	61,92
	Dermis	137,50	14,15
DP07 19209	Epidermis	125,40	32,76
	Dermis	124,50	14,71
DP07-6221	Epidermis	131,80	24,47
	Dermis	137,00	33,25
DP07-8218	Epidermis	141,60	33,01
	Dermis	114,00	5,09
DP845-07	Epidermis	128,60	69,24
	Dermis	119,50	45,01
DP07-7169	Epidermis	133,60	39,04
	Dermis	117,00	17,22
DP07-8617	Epidermis	133,00	17,18
	Dermis	120,00	11,85
DP254-07	Epidermis	112,00	2,81
	Dermis	99,00	2,15
DP07-6294	Epidermis	152,20	86,86
	Dermis	129,33	26,12
DP07-8786	Epidermis	142,00	33,10
	Dermis	134,00	11,72
DP06-11523	Epidermis	153,00	73,08
	Dermis	133,00	58,10
DP07-6295	Epidermis	135,20	89,88
	Dermis	134,67	71,41
DP07-1826	Epidermis	122,40	66,22
	Dermis	129,00	45,75
DP07-5446	Epidermis	148,00	87,19
	Dermis	137,33	66,14
DP07-4746	Epidermis	120,80	60,87
	Dermis	125,67	37,42
DP08 15669	Epidermis	125,20	58,26
	Dermis	127,67	39,88
DP07-1905	Epidermis	104,00	0,61
	Dermis	116,00	8,46
DP07-1157	Epidermis	99,20	75,79

	Dermis	116,33	46,76
DP07-18707	Epidermis	123,20	81,76
	Dermis	101,00	48,73

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Derm Path Number	Area	Intensity	Percentage(%)
DP07-2010	Epidermis	130,00	63,56
	Dermis	125,50	20,79
DP07-3486	Epidermis	111,00	22,16
	Dermis	121,60	68,32
DP07-2495	Epidermis	123,00	26,40
	Dermis	112,80	51,19
DP07-1561	Epidermis	117,00	16,96
	Dermis	111,40	88,89
DP07-5876	Epidermis	100,50	39,31
	Dermis	144,80	88,87
DP07-5878	Epidermis	117,00	18,37
	Dermis	137,20	76,12
DP08-541	Epidermis	124,00	18,89
	Dermis	135,80	84,70
DP08-2678	Epidermis	145,50	26,59
	Dermis	124,80	71,37
DP07 10082-A	Epidermis	117,50	24,66
	Dermis	127,00	87,04
DP07 17053	Epidermis	113,00	15,38
	Dermis	129,20	81,49
DP07 17250	Epidermis	109,50	17,84
	Dermis	132,20	87,85
DP06-2953	Epidermis	117,00	13,08
	Dermis	161,80	88,30
DP06-2974	Epidermis	102,50	22,17
	Dermis	119,20	73,34
DP07 19209	Epidermis	116,50	15,25
	Dermis	130,00	80,24
DP07-8218	Epidermis	112,00	18,61
	Dermis	125,00	15,99

DP845-07	Epidermis	111,40	72,24
	Dermis	99,00	9,82
DP07-7169	Epidermis	109,80	76,35
	Dermis	97,50	13,10
DP07-8617	Epidermis	153,60	87,12
	Dermis	115,50	18,57
DP254-07	Epidermis	166,40	91,09
	Dermis	127,50	16,74
DP07-6294	Epidermis	156,00	85,33
	Dermis	110,50	39,65
DP07-8786	Epidermis	151,20	91,24
	Dermis	119,50	34,43
DP06-11523	Epidermis	162,40	96,92
	Dermis	108,00	39,43
DP06-11523	Epidermis	142,20	64,30
	Dermis	122,00	16,40
DP07-6295	Epidermis	144,60	81,41
	Dermis	111,50	22,08
DP07-5446	Epidermis	145,20	40,62
	Dermis	132,50	23,53
DP07-1826	Epidermis	189,40	88,38
	Dermis	140,50	28,73
DP08-15669	Epidermis	106,40	36,97
	Dermis	126,50	24,34
DP07-4746	Epidermis	190,40	91,26
	Dermis	123,50	15,68
DP07 17053	Epidermis	125,20	40,21
	Dermis	135,50	17,42
DP07-1905	Epidermis	137,20	68,27
	Dermis	116,00	12,85
DP07-1157	Epidermis	121,20	11,60
	Dermis	129,50	26,25
DP07-18707	Epidermis	95,20	14,05
	Dermis	138,00	16,72

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Derm Path Number	Area	Intensity	Percentage (%)
DP07-2010	Epidermis	125,50	1,67
	Dermis	141,40	16,63
DP07 17250	Epidermis	114,00	0,63
	Dermis	141,60	12,38
DP07 17053	Epidermis	103,00	1,00
	Dermis	125,40	12,25
DP07 10082-A	Epidermis	133,50	2,06
	Dermis	130,20	11,83
DP08-2678	Epidermis	110,00	0,65
	Dermis	124,80	9,54
DP08-541	Epidermis	110,00	4,98
	Dermis	118,80	8,67
DP06-2974	Epidermis	108,50	1,41
	Dermis	126,20	11,07
DP07-1561	Epidermis	124,00	0,07
	Dermis	120,00	14,49
DP07-2495	Epidermis	118,50	0,35
	Dermis	137,60	16,42
DP07-3486	Epidermis	115,00	1,47
	Dermis	127,60	16,33
DP07-5876	Epidermis	101,50	0,35
	Dermis	129,00	13,46
DP07-6294	Epidermis	129,00	1,44
	Dermis	130,20	12,21
DP07-8786	Epidermis	101,00	0,15
	Dermis	128,20	8,82
DP254-07	Epidermis	73,00	0,04
	Dermis	94,00	4,62
DP07-8617	Epidermis	99,50	0,41
	Dermis	127,20	12,73
DP854-07	Epidermis	125,50	1,53
	Dermis	134,80	23,36
DP07-7169	Epidermis	122,50	2,02
	Dermis	132,40	8,10
DP2619-06	Epidermis	105,50	0,28

	Dermis	133,00	16,23
DP07-8218	Epidermis	128,50	0,21
	Dermis	116,40	9,83
DP07-6221	Epidermis	101,00	0,13
	Dermis	131,40	10,31
DP07-6295	Epidermis	103,00	4,95
	Dermis	135,00	25,58
DP07-1826	Epidermis	122,00	1,58
	Dermis	113,60	11,45
DP07-5446	Epidermis	148,50	13,86
	Dermis	135,80	21,06
DP07-4746	Epidermis	116,00	3,84
	Dermis	141,40	19,01
DP08 15669	Epidermis	110,50	0,44
	Dermis	135,80	26,79
DP07 19209	Epidermis	103,50	0,31
	Dermis	129,20	5,31
DP06-11523	Epidermis	119,00	2,91
	Dermis	123,00	9,45
DP06 2953	Epidermis	0,00	0,00
	Dermis	128,80	13,52
DP07-1905	Epidermis	105,00	0,87
	Dermis	122,60	7,98
DP07-1157	Epidermis	120,50	1,45
	Dermis	127,20	14,17
DP07-18707	Epidermis	118,00	0,74
	Dermis	122,00	9,67

CD54

Derm Path Number	Area of Interest	Intensity	Percentage (%)
DP06 2953	Epidermis	147,50	18,71
	Dermis	140,00	19,19
DP06-2974	Epidermis	124,50	19,02
	Dermis	144,00	36,48

DP06-11523	Epidermis	118,50	9,87
	Dermis	134,00	30,61
DP2619-06	Epidermis	141,00	18,04
	Dermis	166,00	74,23
DP07-1157	Epidermis	120,50	48,82
	Dermis	159,50	54,79
DP07-1561	Epidermis	138,50	23,03
	Dermis	152,00	72,96
DP07-1826	Epidermis	117,00	35,53
	Dermis	149,00	49,93
DP07-1905	Epidermis	118,50	8,62
	Dermis	136,50	35,65
DP07-2010	Epidermis	151,50	55,02
	Dermis	157,50	32,86
DP07-2495	Epidermis	146,50	28,18
	Dermis	164,00	66,04
DP07-3486	Epidermis	150,00	28,84
	Dermis	167,00	53,81
DP07-4746	Epidermis	156,00	86,26
	Dermis	166,50	92,26
DP07-5446	Epidermis	158,00	42,43
	Dermis	171,00	66,79
DP07-5876	Epidermis	124,00	23,36
	Dermis	153,50	65,28
DP07-5878	Epidermis	134,00	28,78
	Dermis	145,00	25,25
DP07-6221	Epidermis	130,50	21,64
	Dermis	144,50	25,26
DP07-6294	Epidermis	112,50	16,61
	Dermis	135,50	20,01
DP07-6295	Epidermis	125,00	35,33
	Dermis	147,50	58,29
DP07-7169	Epidermis	168,00	54,04
	Dermis	146,00	24,97
DP07-8218	Epidermis	111,00	11,00
	Dermis	133,00	59,54

DP07-8617	Epidermis	127,50	10,79
	Dermis	158,00	57,18
DP08-8786	Epidermis	133,00	17,74
	Dermis	150,50	35,54
DP07 10082-A	Epidermis	150,50	64,76
	Dermis	161,50	49,85
DP07 17053	Epidermis	130,50	11,82
	Dermis	153,50	28,98
DP07 17250	Epidermis	131,50	35,27
	Dermis	160,50	46,94
DP07 18707	Epidermis	139,50	38,28
	Dermis	142,00	48,50
DP07 19209	Epidermis	116,00	14,12
	Dermis	141,50	29,84
DP254-07	Epidermis	137,00	24,82
	Dermis	147,50	47,18
DP854-07	Epidermis	123,00	32,60
	Dermis	153,50	77,36
DP08-541	Epidermis	125,00	45,40
	Dermis	138,50	36,33
DP08-2678	Epidermis	145,50	26,72
	Dermis	157,00	59,48
DP08 15669	Epidermis	115,50	41,42
	Dermis	144,00	54,44

CD20			
Derm Path Number	**Area**	**Intensity**	**Percentage (%)**
DP06 11523	Epidermis	119,00	4,65
	Dermis	114,20	5,90
DP07-8786	Epidermis	122,00	0,18
	Dermis	116,40	1,91
DP07-6294	Epidermis	139,00	1,08
	Dermis	117,60	4,25
DP254-07	Epidermis	122,00	0,19
	Dermis	126,00	5,92
DP07-8619	Epidermis	123,33	0,16

	Dermis	123,60	1,59
DP06 2953	Epidermis	0,00	0,00
	Dermis	124,20	6,48
DP06 2974	Epidermis	123,33	1,37
	Dermis	116,20	3,12
DP2619-06	Epidermis	116,67	0,21
	Dermis	117,00	2,52
DP07-1561	Epidermis	100,33	0,51
	Dermis	118,20	2,13
DP07-1826	Epidermis	111,00	0,59
	Dermis	126,20	2,02
DP07-2010	Epidermis	114,00	0,94
	Dermis	148,40	27,28
DP07-2495	Epidermis	114,00	1,02
	Dermis	120,20	3,07
DP07-3486	Epidermis	129,00	0,94
	Dermis	114,20	2,98
DP07-4746	Epidermis	103,33	2,65
	Dermis	116,00	3,16
DP07-5446	Epidermis	143,00	18,60
	Dermis	120,40	6,28
DP07-5876	Epidermis	89,67	0,20
	Dermis	125,60	7,79
DP07-5878	Epidermis	101,33	0,16
	Dermis	112,60	2,09
DP07-6221	Epidermis	115,00	0,58
	Dermis	125,60	3,40
DP07-6295	Epidermis	96,67	0,81
	Dermis	106,00	1,33
DP07-7169	Epidermis	128,67	1,00
	Dermis	125,80	4,37
DP07-8128	Epidermis	108,67	0,17
	Dermis	112,60	2,64
DP07-8617	Epidermis	129,33	0,40
	Dermis	136,00	3,07
DP07 10082A	Epidermis	127,33	1,60

	Dermis	114,00	4,23
DP07 17053	Epidermis	132,00	1,04
	Dermis	124,20	5,37
DP07 17250	Epidermis	131,00	0,75
	Dermis	123,80	4,95
DP07 19209	Epidermis	101,33	0,25
	Dermis	116,80	2,96
DP854-07	Epidermis	104,00	0,44
	Dermis	131,60	5,28
DP08-541	Epidermis	112,67	5,00
	Dermis	118,60	3,20
DP08-2678	Epidermis	114,67	0,24
	Dermis	121,00	3,10
DP08 15669	Epidermis	101,33	0,38
	Dermis	108,40	2,68
DP07-1905	Epidermis	131,33	1,00
	Dermis	109,80	2,79
DP07-1157	Epidermis	98,33	0,48
	Dermis	123,00	2,57
DP07-18707	Epidermis	116,67	1,94
	Dermis	130,40	5,58

CD11b

Derm Path Number	**Area of Interest**	**Intensity**	**Percentage (%)**
DP06-2953	Epidermis	140,00	3,73
	Dermis	150,00	11,06
DP06-2974	Epidermis	106,00	0,68
	Dermis	116,00	2,79
DP2619-06	Epidermis	118,50	0,29
	Dermis	114,50	2,26
DP07-1561	Epidermis	126,00	1,05
	Dermis	134,50	3,37
DP07-2010	Epidermis	130,00	2,05
	Dermis	127,50	2,94

DP07-2495	Epidermis	141,50	0,92
	Dermis	126,50	3,03
DP07-3486	Epidermis	126,50	1,20
	Dermis	133,50	4,44
DP07-5876	Epidermis	132,50	0,66
	Dermis	139,50	2,82
DP07-5878	Epidermis	159,00	2,53
	Dermis	138,00	3,28
DP07-6221	Epidermis	152,50	13,07
	Dermis	119,00	2,01
DP07-6294	Epidermis	120,50	0,90
	Dermis	120,00	2,34
DP07-7169	Epidermis	140,00	0,68
	Dermis	139,50	0,95
DP07-8218	Epidermis	130,50	0,19
	Dermis	110,00	1,51
DP07-8617	Epidermis	124,00	0,70
	Dermis	135,50	9,27
DP07-8786	Epidermis	102,50	0,33
	Dermis	121,50	2,20
DP07 10082-A	Epidermis	106,50	0,65
	Dermis	135,00	4,16
DP07-17053	Epidermis	116,50	0,97
	Dermis	119,50	1,85
DP07 17250	Epidermis	122,00	0,81
	Dermis	112,50	1,84
DP07 19209	Epidermis	111,50	0,38
	Dermis	118,50	2,08
DP254-07	Epidermis	136,00	0,55
	Dermis	120,00	3,87
DP845-07	Epidermis	124,00	1,10
	Dermis	140,50	4,29
DP08-541	Epidermis	135,50	3,86
	Dermis	134,00	3,36
DP08-2678	Epidermis	137,50	1,62
	Dermis	126,00	3,38

DP06-11523	Epidermis	120,50	2,60
	Dermis	120,00	9,94
DP07-1157	Epidermis	125,50	1,96
	Dermis	131,50	5,30
DP07-1826	Epidermis	136,00	3,10
	Dermis	122,50	6,81
DP07-1905	Epidermis	134,00	3,15
	Dermis	99,50	3,90
DP07-4746	Epidermis	170,00	17,07
	Dermis	158,00	35,01
DP075446	Epidermis	154,00	11,92
	Dermis	119,00	10,27
DP07-6295	Epidermis	101,50	0,57
	Dermis	107,50	6,03
DP07-18707	Epidermis	118,00	5,71
	Dermis	126,50	6,71
DP08-15669	Epidermis	126,50	0,79
	Dermis	114,50	5,56

CD8

Derm Path Number	Area	Intensity	Percentage (%)
DP06 2953	Epidermis	137,40	3,12
	Dermis	142,00	4,67
DP06 2974	Epidermis	113,00	1,47
	Dermis	125,00	3,72
DP06 11523	Epidermis	135,60	2,33
	Dermis	103,50	2,76
DP2619-06	Epidermis	122,00	0,30
	Dermis	152,00	6,09
DP07-1561	Epidermis	70,40	2,06
	Dermis	138,50	15,56
DP07-1826	Epidermis	127,00	0,29
	Dermis	139,50	8,78
DP07-2010	Epidermis	103,40	0,90
	Dermis	168,50	20,86
DP07-2495	Epidermis	106,00	0,60

	Dermis	142,50	8,40
DP07-3486	Epidermis	141,80	4,79
	Dermis	145,00	15,05
DP07-4746	Epidermis	130,60	1,87
	Dermis	154,00	6,38
DP07-5446	Epidermis	139,60	11,50
	Dermis	161,00	13,42
DP07-5878	Epidermis	127,60	0,65
	Dermis	142,50	8,31
DP07-5876	Epidermis	111,80	0,37
	Dermis	149,50	16,70
DP07-6221	Epidermis	95,20	0,55
	Dermis	128,00	3,81
DP07-6294	Epidermis	102,40	1,09
	Dermis	155,50	13,57
DP07-6295	Epidermis	99,20	0,18
	Dermis	139,00	5,50
DP07-7169	Epidermis	113,80	1,90
	Dermis	134,00	13,47
DP07-8218	Epidermis	98,60	0,30
	Dermis	125,50	5,03
DP07-8617	Epidermis	116,60	0,14
	Dermis	155,00	12,84
DP07-8786	Epidermis	102,00	0,45
	Dermis	146,50	17,96
DP07 100082-A	Epidermis	147,60	6,59
	Dermis	137,00	7,70
DP07-17053	Epidermis	131,40	2,59
	Dermis	138,50	9,27
DP07-17250	Epidermis	131,80	3,59
	Dermis	119,50	4,01
DP07-19209	Epidermis	90,80	1,98
	Dermis	109,50	3,82
DP254-07	Epidermis	106,00	1,55
	Dermis	110,50	1,42
DP854-07	Epidermis	107,60	0,91

	Dermis	140,00	4,39
DP08-541	Epidermis	140,40	7,74
	Dermis	154,50	4,68
DP08-2678	Epidermis	119,00	0,53
	Dermis	157,50	13,69
DP08-15669	Epidermis	109,40	0,19
	Dermis	136,00	2,72
DP07-1905	Epidermis	101,00	0,76
	Dermis	127,50	11,99
DP07-1157	Epidermis	101,00	0,54
	Dermis	147,50	6,66
DP07-18707	Epidermis	104,00	0,71
	Dermis	118,50	5,32

CD4

Derm Path Number	Area	Intensity	Percentage
DP07 19209	Epidermis	113,00	0,17
	Dermis	124,60	3,63
DP07-6221	Epidermis	106,67	0,32
	Dermis	119,20	3,49
DP07-8218	Epidermis	121,00	0,21
	Dermis	114,00	5,01
DP845-07	Epidermis	112,33	0,27
	Dermis	112,60	3,78
DP07-7169	Epidermis	119,00	1,21
	Dermis	108,20	4,10
DP07-8617	Epidermis	123,00	0,86
	Dermis	125,40	4,07
DP254-07	Epidermis	134,67	0,04
	Dermis	128,40	17,86
DP07-6294	Epidermis	122,33	0,89
	Dermis	128,60	6,96
DP07-8786	Epidermis	115,67	0,04
	Dermis	122,40	3,36
DP07-2010	Epidermis	117,67	0,43
	Dermis	130,60	11,46

DP07-3486	Epidermis	130,00	1,31
	Dermis	131,20	10,01
DP07-2495	Epidermis	118,00	1,11
	Dermis	127,80	8,54
DP07-1561	Epidermis	116,67	0,56
	Dermis	119,40	7,01
DP07-5876	Epidermis	118,33	0,89
	Dermis	126,40	5,97
DP07-5878	Epidermis	112,33	0,89
	Dermis	121,00	4,57
DP08-541	Epidermis	143,67	6,70
	Dermis	134,80	8,00
DP08-2678	Epidermis	120,33	0,59
	Dermis	129,00	11,41
DP07 10082-A	Epidermis	104,00	0,28
	Dermis	121,80	9,06
DP07 17053	Epidermis	133,33	1,40
	Dermis	125,80	5,86
DP07 17250	Epidermis	121,33	71,35
	Dermis	115,80	18,57
DP06-2953	Epidermis	134,33	8,56
	Dermis	136,00	5,75
DP06-11523	Epidermis	133,67	1,54
	Dermis	126,20	3,25
DP07-6295	Epidermis	106,00	0,35
	Dermis	116,80	2,67
DP07-1826	Epidermis	113,00	0,33
	Dermis	116,60	2,13
DP07-5446	Epidermis	147,00	3,94
	Dermis	122,00	7,81
DP07-4746	Epidermis	123,33	2,84
	Dermis	120,40	4,98
DP08 15669	Epidermis	100,00	0,11
	Dermis	115,80	3,14
DP07-1905	Epidermis	109,33	0,38
	Dermis	112,80	4,97

DP07-1157	Epidermis	115,33	0,43
	Dermis	123,60	16,11
DP07-18707	Epidermis	116,00	0,61
	Dermis	119,00	3,98
DP07-18707	Epidermis	99,00	1,37
	Dermis	110,40	3,71

CD1a

Derm Path Number	Area	Intensity	Percentage (%)
DP07-2010	Epidermis	139,00	22,38
	Dermis	120,00	4,06
DP07-3486	Epidermis	144,00	4,74
	Dermis	134,33	11,60
DP07-2495	Epidermis	141,00	15,18
	Dermis	134,83	12,94
DP07-1561	Epidermis	127,00	10,30
	Dermis	114,33	4,45
DP07-5876	Epidermis	133,00	4,25
	Dermis	122,33	5,78
DP07-5878	Epidermis	128,00	9,33
	Dermis	124,67	8,96
DP08-541	Epidermis	128,00	21,15
	Dermis	131,67	10,18
DP08-2678	Epidermis	147,00	12,36
	Dermis	137,33	12,82
DP07-10082A	Epidermis	133,67	9,34
	Dermis	113,80	6,26
DP07-17053	Epidermis	108,00	7,00
	Dermis	122,17	8,46
DP07-17250	Epidermis	141,00	10,92
	Dermis	119,50	3,57
DP07-7169	Epidermis	0,00	0,00
	Dermis	122,17	6,00
DP07-8617	Epidermis	132,00	2,55
	Dermis	125,17	8,45

DP07-254	Epidermis	136,00	3,73
	Dermis	126,83	13,60
DP07-6294	Epidermis	123,00	1,85
	Dermis	115,00	4,36
DP07-8786	Epidermis	136,00	2,85
	Dermis	130,67	6,06
DP06-2974	Epidermis	135,33	6,31
	Dermis	121,17	8,81
DP07-19209	Epidermis	137,33	7,47
	Dermis	109,17	6,48
DP07-6221	Epidermis	113,33	3,76
	Dermis	108,67	2,63
DP07-8218	Epidermis	98,67	0,21
	Dermis	110,50	4,61
DP06-2619	Epidermis	124,33	2,95
	Dermis	121,00	7,97
DP07-854	Epidermis	132,67	14,06
	Dermis	119,50	11,77
DP06-11523	Epidermis	133,33	10,47
	Dermis	121,83	8,69
DP07-6295	Epidermis	116,33	2,27
	Dermis	125,17	8,40
DP07-1826	Epidermis	123,00	8,44
	Dermis	117,67	6,85
DP07-5446	Epidermis	145,00	24,90
	Dermis	113,17	7,44
DP07-4746	Epidermis	137,33	15,13
	Dermis	124,17	5,33
DP08-15669	Epidermis	88,33	0,77
	Dermis	116,33	4,98
DP07-18707	Epidermis	124,33	5,47
	Dermis	123,67	10,27
DP07-1157	Epidermis	133,00	9,28
	Dermis	106,83	2,06
DP07-1905	Epidermis	134,00	2,64
	Dermis	116,33	5,79

DP07-254	Epidermis	141,67	3,47
	Dermis	119,20	14,83

Stat3

Derm Path Number	Area of Interest	Intensity	Percentage (%)
DP07 19209	Epidermis	138,00	84,04
	Dermis	127,60	42,97
DP07-6221	Epidermis	107,00	54,94
	Dermis	114,60	43,39
DP07-8218	Epidermis	143,00	94,62
	Dermis	123,00	41,18
DP07-7619	Epidermis	132,00	71,91
	Dermis	125,20	47,78
DP07-8617	Epidermis	131,00	91,91
	Dermis	136,80	49,78
DP254-07	Epidermis	116,00	46,48
	Dermis	129,40	49,04
DP07-6294	Epidermis	137,33	95,75
	Dermis	106,00	39,73
DP07-8786	Epidermis	115,67	66,98
	Dermis	120,20	38,89
DP07-2010	Epidermis	151,67	98,66
	Dermis	136,00	56,46
DP07-1561	Epidermis	146,67	91,71
	Dermis	135,60	54,18
DP07-2495	Epidermis	150,00	95,99
	Dermis	133,80	51,09
DP07-3486	Epidermis	170,00	98,52
	Dermis	152,20	59,01
DP07-5876	Epidermis	123,67	74,09
	Dermis	133,00	47,09
DP07-5878	Epidermis	162,33	95,01
	Dermis	136,80	55,76
DP08-541	Epidermis	142,33	94,84
	Dermis	129,40	49,09

DP08-2678	Epidermis	149,67	86,50
	Dermis	145,00	48,23
DP07 10082-A	Epidermis	146,00	97,39
	Dermis	141,20	42,87
DP07 17053	Epidermis	173,33	96,49
	Dermis	141,40	46,94
DP07 17250	Epidermis	186,67	96,66
	Dermis	135,00	40,48
DP08 15669	Epidermis	175,67	98,88
	Dermis	147,80	57,99
DP07-4746	Epidermis	165,67	98,88
	Dermis	118,80	50,93
DP07-5446	Epidermis	172,67	91,83
	Dermis	146,40	57,87
DP07-1826	Epidermis	159,67	92,29
	Dermis	135,20	56,87
DP07-6295	Epidermis	152,00	96,99
	Dermis	155,00	60,44
DP06-11523	Epidermis	155,00	94,63
	Dermis	132,20	48,27
DP845-07	Epidermis	158,00	95,77
	Dermis	154,80	54,67
DP06-2974	Epidermis	144,00	88,89
	Dermis	136,40	55,73
DP07-1905	Epidermis	157,33	97,86
	Dermis	136,80	56,48
DP07-1157	Epidermis	136,33	99,05
	Dermis	152,60	60,33
DP07-1157	Epidermis	128,67	98,54
	Dermis	138,40	58,98
DP07-18707	Epidermis	158,33	97,85
	Dermis	109,60	54,65

pHER1

Derm Path Number	Area of Interest	Intensity	Percentage (%)

DP07-2010	Epidermis	142,00	58,66
	Dermis	135,50	57,10
DP07-1564	Epidermis	116,40	6,54
	Dermis	127,00	38,37
DP07-5876	Epidermis	125,80	4,27
	Dermis	129,50	38,91
DP07-5878	Epidermis	123,80	20,95
	Dermis	133,00	28,00
DP08-541	Epidermis	130,20	34,95
	Dermis	135,50	27,55
DP08-2678	Epidermis	114,60	3,26
	Dermis	130,00	29,61
DP07 10082	Epidermis	111,80	12,18
	Dermis	130,00	34,84
DP07 17053	Epidermis	113,00	3,95
	Dermis	122,50	36,81
DP07 17250	Epidermis	131,00	19,36
	Dermis	139,50	17,20
DP06-2953	Epidermis	139,60	13,88
	Dermis	134,00	43,46
DP06-2974	Epidermis	123,80	18,09
	Dermis	127,00	21,79
DP07 19209	Epidermis	117,40	8,79
	Dermis	128,00	24,86
DP07-6221	Epidermis	114,40	0,57
	Dermis	111,00	28,50
DP07-8218	Epidermis	111,60	5,44
	Dermis	118,50	20,74
DP2619-06	Epidermis	140,80	34,63
	Dermis	131,00	17,85
DP845-07	Epidermis	114,20	4,30
	Dermis	115,50	32,59
DP07-7169	Epidermis	113,40	7,80
	Dermis	126,50	11,44
DP254-07	Epidermis	128,00	2,14
	Dermis	146,00	31,31

DP07-8617	Epidermis	130,40	8,89
	Dermis	126,00	22,88
DP07-6294	Epidermis	124,00	8,03
	Dermis	134,50	44,67
DP07-8786	Epidermis	109,60	1,24
	Dermis	127,50	47,68
DP07-6295	Epidermis	104,80	93,34
	Dermis	121,50	79,48
DP07-1826	Epidermis	105,20	77,35
	Dermis	121,50	70,43
DP07-5446	Epidermis	127,80	75,03
	Dermis	131,00	84,23
DP07-4746	Epidermis	105,00	79,95
	Dermis	111,50	84,35
DP08 15669	Epidermis	110,20	98,70
	Dermis	118,00	88,10
DP07-1905	Epidermis	104,60	0,64
	Dermis	118,00	6,12
DP07-1157	Epidermis	121,00	0,80
	Dermis	118,50	3,57
DP07-18707	Epidermis	112,20	3,33
	Dermis	127,50	6,38

APPENDIX B

BIOSTATISTICAL DATA FROM THE SAS SYSTEM STATISTICAL SOFTWARE (SAS INSTITUTE INC. 2007. SAS ONLINEDOC® 9.2. CARY, NC: SAS INSTITUTE INC.)

KRT1		drug 1	drug 1		drug 2	drug 2		drug 3	drug 3		drug 4	drug 4		total		p-value across 4 drugs	p-value, drug 3 vs 1, 2, 4	pairwise significance
		mean	sem		mean	sem		mean	sem		mean	sem		mean	sem			
	epidermis	127,6	9,7		131,0	7,1		127,2	8,0		126,2	7,6		128,0	4,1	0,97	0,91	none
	dermis	4,7	0,5		4,8	0,6		4,2	0,7		4,3	0,7		4,5	0,3	0,90	0,65	none
	total	66,1	5,0		67,9	3,6		65,7	4,1		65,2	3,8				0,96	0,89	none
														p<0.0001 between skin layers	p=0.98 for interaction			

HLA-DR		drug 1	drug 1		drug 2	drug 2		drug 3	drug 3		drug 4	drug 4		total		p-value across 4 drugs	p-value, drug 3 vs 1, 2, 4	pairwise significance
		mean	sem		mean	sem		mean	sem		mean	sem		mean	sem			
	epidermis	17,8	2,3		20,0	5,6		13,9	2,3		13,3	1,3		16,3	1,7	0,29	0,33	none
	dermis	34,2	8,0		38,1	11,8		25,7	5,7		25,1	4,0		30,8	4,0	0,59	0,38	none
	total	26,0	4,4		29,1	8,0		19,8	3,3		19,2	2,4				0,40	0,29	none
														p=0.0003 between skin layers	p=0.89 for interaction			
ERK1		drug 1	drug 1		drug 2	drug 2		drug 3	drug 3		drug 4	drug 4		total		p-value across 4 drugs	p-value, drug 3 vs 1, 2, 4	pairwise significance
		mean	sem		mean	sem		mean	sem		mean	sem		mean	sem			
	epidermis	79,7	12,8		79,1	9,4		84,9	12,2		83,8	12,3		81,9	5,9	0,98	0,78	none
	dermis	52,2	6,2		54,0	3,8		65,3	3,0		63,7	5,9		58,8	2,5	0,08	0,06	4
	total	65,9	9,0		66,6	6,3		75,1	7,0		73,7	8,8				0,75	0,46	none
														p<0.0001 between skin layers	p=0.90 interaction			

K16		**drug 1**	**drug 1**		**drug 2**	**drug 2**		**drug 3**	**drug 3**		**drug 4**	**drug 4**		**total**		**p-value across 4 drugs**	**p-value, drug 3 vs 1, 2, 4**	**pairwise significance**
		mean	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**			
	epidermis	69,8	16,8		63,7	13,7		93,1	10,1		104,9	13,0		82,9	6,8	0,13	0,31	5
	dermis	39,1	6,9		46,3	6,8		31,4	3,6		43,6	5,3		40,1	2,9	0,14	0,032	none
	total	54,5	8,9		55,0	9,5		62,3	6,2		74,2	6,9				0,26	0,90	none
														p<0.0001 between skin layers	p=0.012 interaction			
Ki67		**drug 1**	**drug 1**		**drug 2**	**drug 2**		**drug 3**	**drug 3**		**drug 4**	**drug 4**		**total**		**p-value across 4 drugs**	**p-value, drug 3 vs 1, 2, 4**	**pairwise significance**
		mean	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**			
	epidermis	16,6	2,7		20,0	5,0		27,9	5,9		35,9	6,6		25,1	2,6	0,046	0,58	3
	dermis	7,8	2,2		9,1	1,6		11,2	1,8		12,1	3,0		10,0	1,1	0,52	0,49	none
	total	12,2	1,5		14,6	2,7		19,6	3,0		24,0	4,6				0,04	0,47	2, 3

													p<0.0001 between skin layers	p=0.11 interaction				
p27		**drug 1**	**drug 1**		**drug 2**	**drug 2**		**drug 3**	**drug 3**		**drug 4**	**drug 4**		**total**		**p-value across 4 drugs**	**p-value, drug 3 vs 1, 2, 4**	**pairwise significance**
		mean	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**			
	epidermis	16,6	3,7		16,5	3,6		18,3	4,5		20,4	3,5		17,8	1,9	0,82	0,89	none
	dermis	19,2	1,6		24,7	2,7		19,4	1,1		29,3	5,1		23,1	1,5	0,10	0,04	none
	total	17,6	2,4		20,6	2,5		18,8	2,1		24,8	3,2				0,33	0,41	none
														p=0.03 between skin layers	p=0.57 interaction			
pAKT		**drug 1**	**drug 1**		**drug 2**	**drug 2**		**drug 3**	**drug 3**		**drug 4**	**drug 4**		**total**		**p-value across 4 drugs**	**p-value, drug 3 vs 1, 2, 4**	**pairwise significance**
		mean	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**			
	epidermis	78,5	17,0		50,8	13,2		123,3	16,4		123,6	11,0		94,0	7,3	0,001	0,043	3, 4, 5
	dermis	37,9	10,5		28,1	7,6		79,2	12,1		56,2	9,9		50,3	5,1	0,008	0,007	2, 4, 5

	total	58,2	12,0		39,5	9,8		101,2	11,9		89,9	7,0				0,0006	0,007	2, 3, 4, 5
														p<0.0001 between skin layers	p=0.10 interaction			
EGFR		**drug 1**	**drug 1**		**drug 2**	**drug 2**		**drug 3**	**drug 3**		**drug 4**	**drug 4**		**total**		**p-value across 4 drugs**	**p-value, drug 3 vs 1, 2, 4**	**pairwise significance**
		mean	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**			
	epidermis	81,3	24,1		77,4	17,7		38,4	11,7		76,3	21,8		68,4	9,7	0,16	0,03	none
	dermis	47,6	16,4		29,0	8,9		99,1	11,4		47,6	10,5		55,8	6,1	0,0006	0,0002	2, 4
	total	64,5	12,8		53,2	7,3		68,7	2,5		61,9	8,7				0,25	0,17	none
														p=0.37 between skin layers	p=0.01 interaction			
CD68		**drug 1**	**drug 1**		**drug 2**	**drug 2**		**drug 3**	**drug 3**		**drug 4**	**drug 4**		**total**		**p-value across 4 drugs**	**p-value, drug 3 vs 1, 2, 4**	**pairwise significance**
		mean	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**			
	epidermis	1,01	0,47		1,19	0,30		2,59	0,83		4,04	2,40		2,20	0,65	0,26	0,67	none

	dermis	14,32	1,31		17,61	3,88		15,55	3,02		21,02	1,94		17,13	1,36	0,059	0,54	3
	total	7,65	0,66		9,40	1,96		9,07	1,67		12,53	1,97				0,13	0,68	3
														p<0.0001 between skin layers	p=0.43 interaction			
CD54		**drug 1**	**drug 1**		**drug 2**	**drug 2**		**drug 3**	**drug 3**		**drug 4**	**drug 4**		**total**		**p-value across 4 drugs**	**p-value, drug 3 vs 1, 2, 4**	**pairwise significance**
		mean	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**			
	epidermis	25,1	4,6		39,9	9,3		45,9	8,5		57,3	13,3		42,1	4,7	0,046	0,62	2, 3
	dermis	57,0	11,6		72,7	9,4		70,6	8,6		93,4	12,7		73,4	5,4	0,23	0,72	3
	total	41,1	6,5		56,3	6,0		58,3	6,5		75,4	11,6				0,07	0,94	3
														p<0.0001 between skin layers	p=0.91 interaction			
CD20		**drug 1**	**drug 1**		**drug 2**	**drug 2**		**drug 3**	**drug 3**		**drug 4**	**drug 4**		**total**		**p-value across 4 drugs**	**p-value, drug 3 vs 1, 2, 4**	**pairwise significance**
		mean	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**			

	epidermis	1,51	0,73		0,78	0,19		1,29	0,66		4,41	3,18		2,09	0,83	0,48	0,46	none
	dermis	4,93	0,78		4,60	0,64		4,44	0,92		8,82	4,57		5,70	1,19	0,80	0,36	none
	total	3,40	0,73		2,70	0,30		2,86	0,54		6,62	2,72				0,45	0,22	none
														p=0.02 between skin layers	p=0.85 interaction			
CD11b		**drug 1**	**drug 1**		**drug 2**	**drug 2**		**drug 3**	**drug 3**		**drug 4**	**drug 4**		**total**		**p-value across 4 drugs**	**p-value, drug 3 vs 1, 2, 4**	**pairwise significance**
		mean	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**			
	epidermis	4,63	2,35		1,54	0,43		1,88	0,64		7,44	3,70		3,87	1,11	0,27	0,11	none
	dermis	6,14	1,97		5,62	1,19		4,23	0,52		12,02	6,29		7,00	1,68	0,37	0,12	none
	total	5,38	1,55		3,58	0,61		3,06	0,43		9,73	4,88				0,28	0,08	none
														p=0.01 between skin layers	p=0.63 interaction			
CD8		**drug 1**	**drug 1**		**drug 2**	**drug 2**		**drug 3**	**drug 3**		**drug 4**	**drug 4**		**total**		**p-value across 4 drugs**	**p-value, drug 3 vs 1, 2, 4**	**pairwise significance**
		mean	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**			

	epidermis	1,37	0,53		1,01	0,26		3,65	1,54		4,01	1,87		2,51	0,62	0,17	0,37	none
	dermis	10,45	3,00		9,89	2,48		11,59	2,72		18,38	2,98		12,58	1,40	0,16	0,68	5
	total	5,91	1,44		5,45	1,23		7,62	1,28		11,19	1,88				0,08	0,95	3, 5
														p<0.0001 between skin layers	p=0.50 interaction			
CD4		**drug 1**	**drug 1**		**drug 2**	**drug 2**		**drug 3**	**drug 3**		**drug 4**	**drug 4**		**total**		**p-value across 4 drugs**	**p-value, drug 3 vs 1, 2, 4**	**pairwise significance**
		mean	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**			
	epidermis	2,29	1,56		0,65	0,19		12,48	10,64		1,96	0,65		4,35	2,69	0,15	0,32	none
	dermis	5,66	0,75		8,75	2,79		9,84	2,15		9,09	1,41		8,34	0,97	0,08	0,41	3
	total	3,97	1,02		4,70	1,36		11,16	6,21		5,53	0,76				0,48	0,31	none
														p=0.11 between skin layers	p=0.19 interaction			
CD1a		**drug 1**	**drug 1**		**drug 2**	**drug 2**		**drug 3**	**drug 3**		**drug 4**	**drug 4**		**total**		**p-value across 4 drugs**	**p-value, drug 3 vs 1, 2, 4**	**pairwise significance**

		mean	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**			
	epidermis	5,01	1,67		7,46	2,36		12,96	2,67		18,40	3,86		10,94	1,38	0,01	0,40	2, 3, 5
	dermis	7,69	1,33		9,33	1,70		9,78	1,52		9,55	1,65		9,09	0,78	0,71	0,61	none
	total	6,35	1,34		8,36	1,54		11,37	1,80		13,98	1,86				0,014	0,38	2, 3, 5
														p=0.23 between skin layers	p=0.049 interaction			
Stat3		**drug 1**	**drug 1**		**drug 2**	**drug 2**		**drug 3**	**drug 3**		**drug 4**	**drug 4**		**total**		**p-value across 4 drugs**	**p-value, drug 3 vs 1, 2, 4**	**pairwise significance**
		mean	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**		**mean**	**sem**			
	epidermis	117,5	16,1		125,4	13,3		137,0	9,3		154,1	4,3		133,5	5,8	0,036	0,69	3, 5
	dermis	52,2	3,3		74,6	3,5		67,0	4,6		74,6	3,4		67,1	1,9	0,0002	0,98	1, 2, 3
	total	84,8	9,0		100,0	8,2		102,0	5,5		114,3	2,8				0,01	0,74	3
														p<0.0001 between skin layers	p=0.12 interaction			

pEGFR		drug 1	drug 1		drug 2	drug 2		drug 3	drug 3		drug 4	drug 4		total		p-value across 4 drugs	p-value, drug 3 vs 1, 2, 4	pairwise significance
		mean	sem		mean	sem		mean	sem		mean	sem		mean	sem			
	epidermis	12,8	6,5		20,1	12,9		28,5	11,0		50,7	14,0		28,0	5,7	0,11	0,96	3
	dermis	38,1	8,1		33,7	11,3		45,1	7,9		60,7	13,1		44,4	5,2	0,42	0,92	none
	total	25,5	4,8		26,9	11,8		36,8	8,9		55,7	12,9				0,17	0,94	3
														p=0.0004 between skin layers	p=0.71 interaction			

Legend:
Drug 1=Cetuximab; Drug 2=Erlotinib; Drug 3=Lapatinib; Drug 4=Panitumumab
Coding for pairwise significance:
1: p<.05 drug 1 vs drug 2; **2**: p<.05 drug 1 vs drug 3; **3**: p<.05 drug 1 vs drug 4; **4**: p<.05 drug 2 vs drug 3;
5: p<.05 drug 2 vs drug 4; **6**:p<.05 drug 3 vs drug 4