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Characterisation of the cutaneous pathology in non-small cell lung cancer (NSCLC) patients treated with the EGFR tyrosine kinase inhibitor erlotinib

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ABSTRACT

Introduction: EGFR inhibitors (EGFRIs) have been shown to be clinically effective in various cancers. Unique skin toxicity is commonly observed with EGFRIs and a correlation between the clinical benefit of EGFRIs and this characteristic rash has been reported. Erlotinib is a potent EGFRI approved for treatment of non-small cell lung cancer (NSCLC) and pancreatic cancer.

Methods: This is the first time in which patients were given increasing doses of an EGFRI to induce a mechanistic rash and study its associated pathology in skin. Biopsies were collected during treatment from both rash-affected and unaffected skin of 23 NSCLC patients and compared with pre-treatment biopsies.

Results: Altered differentiation of appendegeal epithelium (hair follicles and sebaceous glands) was remarkable in both affected and unaffected skin, although epidermal growth was not significantly reduced. A predominantly mononuclear leucocyte infiltrate was detected in the interfollicular dermis or around skin appendages. This infiltrate included TRAIL-positive cells with a dendritic cell (DC) morphology, although T-cells, antigen-presenting DCs and macrophages were also evident. This is the first report showing the involvement of a dendritic cell subtype with EGFRI skin toxicity.

Conclusions: Altered differentiation of pilosebaceous epithelium is evident in both rash-affected and unaffected skin and constitutes the primary process of EGFRI in human skin. We propose that this eventually triggers inflammation and the EGFRI rash. TRAIL-positive inflammatory cells could link rash development and immune-triggered apoptosis of epithelial cells, including those of underlying carcinomas.

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1. Introduction

EGFR-targeted drugs emerged as robust therapeutic agents against various malignancies.[1] Unlike standard chemotherapy that acts on replicating cells, EGFR inhibitors (EGFRIs) target only cells expressing the cognate receptor.[2–5] Therefore, these drugs have different pharmacologic actions and systemic side-effects compared with standard chemotherapeutic drugs.[6] Erlotinib (Tarceva®; OSI-774) is a potent, selective small-molecule EGFRI approved for NSCLC and pancreatic cancer.[7,8]

The increasing clinical usage of EGFRIs has led to the identification of commonly occurring side effects which are most evident in tissues dependent on EGFR signalling for normal function, such as the skin.[1] Unique skin toxicity was reported as follicular, 'acneiform' rash.[1,9–12] Although this rash resembles acne vulgaris, it is predominantly pustular, not associated with comedones, may involve different body areas and is pathologically distinct from acne vulgaris.[1,9,13] Most patients exhibit mild skin symptoms, but moderate to severe toxicities, leading to dose adjustment or interruption of therapy, occur in 8–17% of patients.[7] The clinical benefit of EGFRIs has been suggested to correlate with severity of this characteristic rash, rendering rash a potential marker of drug activity.[13,14] The optimum management of this rash remains somewhat anecdotal, as the rash does not typically respond to anti-acne agents, but sometimes to anti-inflammatory, antibiotic and immunomodulatory agents.[1,13,15–17]

The high frequency of cutaneous manifestations with EGFRIs may reflect the function of EGFR in epithelial or mesenchymal cells associated with the epidermis, sweat gland apparatus, hair follicle and periungual tissues.[18–20] Studies in mice lacking EGFR at these sites showed a central role of EGFR in normal differentiation and development of the hair follicle, as these mice presented disorganised hair follicle phenotypes.[21–23] While EGF attenuates hair follicle growth and differentiation, it has pleiotropic effects on cultured keratinocytes.[24,25] *In vitro* models using organ cultures established a role for EGF and TGF-α in the regulation of sebocyte and keratinocyte differentiation in human pilosebaceous unit.[26]

Cutaneous inflammation in several disease models is linked to inflammatory dendritic cells (DCs),[27] where myeloid DCs are greatly increased over background resident DCs in the skin. These DCs accumulate in parallel with T-cells, have T-cell stimulatory activity and produce direct inflammatory mediators, such as inducible nitric oxide (iNOS), TNF-α and chemokines.[27]

Although prior EGFRI studies reported skin findings, these did not usually integrate cutaneous biology and histology, possibly precluding a clear understanding of patho-physiological mechanisms of these side effects, and adequate treatment guidelines.[1] The present study is the first in which patients with advanced NSCLC were given escalating doses of EGFRI in order to induce a mechanistic rash for studying its associated pathology. We identified profound alterations in growth and differentiation of appendegeal epithelium, including sebaceous glands, in affected and unaffected skin. Inflammation was associated with a mononuclear infiltrate, including TRAIL-positive cells with a DC morphology. This cell type has been previously shown to have cytotoxic function in mouse and human cancers.[28,29]

2. Material and methods

A multi-centre, open-label, phase 2 study (NCT00072631) was conducted to determine the feasibility of intrapatient dose escalation to induce a characteristic target rash (TR) and evaluate the effect on objective tumour response rate in patients with advanced NSCLC. TR was defined as a grade 2 rash, symptomatic, albeit tolerable rash, which required intervention with oral minocycline.

2.1. Patient data

Patients were 18 years or older with histologically confirmed stage IIIB/IV NSCLC and previously treated with at least one

Table 1 – Summary of rash characteristics.

	Erlotinib (N = 42)
	No. (%)
Description of rash	
None reported	1 (2)
Rosacea-like	12 (29)
Acneiform-like	40 (95)
Punctate-like	7 (17)
Area(s) of body involved	
Face	37 (88)
Head/neck	34 (81)
Back/chest	37 (88)
Extremities	28 (67)
Other	6 (14)
Action taken	
None	9 (21)
Dose held	12 (29)
Dose modified	23 (55)
Treatment administered	28 (67)
Withdrawn from study	0 (0)
Characteristics present	
Erythema	32 (76)
Pruritus	28 (67)
Papules	32 (76)
Induration	9 (21)
Pustules	35 (83)
Desquamation	16 (38)
Urticaria	11 (26)
Pain	10 (24)
Body surface area involved (%)	
<25%	12 (29)
25–50%	21 (50)
>50%	8 (19)
Seriousness	
Yes	1 (2)
No	40 (95)

chemotherapy regimen. The study was approved by the Institutional Review Board.

2.2. *Drug administration*

Erlotinib monotherapy was administered at 150 mg/day for 21 days with subsequent dose escalations unless intolerable rash despite treatment with oral minocycline, or if other, dose-limiting toxicity (DLT) occurred.

2.3. *Evaluation of skin manifestations*

Biopsies from normal-appearing skin were obtained before erlotinib treatment. At the first appearance of TR the resulting skin rash was characterised by a thorough analysis of clinical pictures and punch biopsies collected from both rash-affected and unaffected areas of the skin. Tissue sections (4 μm) were stained with haematoxylin and eosin (H&E), and additional analyses were performed on biopsy material including: Giemsa stain, Gram stain and PAS for fungus. For assessment of rash skin immune infiltrate, mouse anti-human monoclonal antibodies were used to the following: CD3, CD1a, CD163, TRAIL, TLR-2, DC-SIGN and CD11c. Detailed patient and drug administration data, analyses and antibody list are given in Supplementary materials.

3. Results

Forty-two patients (28 males, 14 females), median age 63 years (range 41–78), participated in the study. The clinical results were previously presented and will be published separately.[30] Forty-one (98%) of 42 enrolled patients experienced rash (Table 1); 24 (59%) of 41 patients with rash experienced a TR and had biopsies performed. Biopsies paired with matching clinical photographs were available from 23 (95%) patients with a TR. For 19 (83%) of these patients, the TR first occurred at the starting 150 mg/day dose. An attempt was made to correlate clinical features of the rash with histopathology.

An inflammatory erythematous papulo-pustular rash, in a follicular pattern, involving the entire face, head, neck, upper trunk and upper extremities was the most common skin manifestation (Table 1). The rash was associated with diffuse telangiectasia (Fig. 1A). Serum crusts were occasionaly noticed, possibly indicating a secondary impetigo infection (Fig. 1B and D). The rash extended to areas not usually involving acne vulgaris, such as the entire head (including the scalp), neck and upper extremities. Fig. 1C shows a representative example of the rash, with extensive erythema and numerous confluent pustules, in a follicular distribution, although most pustules are not associated with terminal hair follicles. The nose is commonly involved (Fig. 1D).

Biopsies taken from affected and unaffected skin were scored with respect to abnormal or deranged differentiation of skin structures and immune infiltration to various regions (Table 2). Certain paired skin biopsies were lacking detectable structures (such as sebaceous or eccrine glands and dermis) to assess alterations or infiltrate (one example in each case), hence the variation in denominator between 22 and 23.

Altered differentiation of epithelial structures, as compared to pre-treatment baseline, was remarkable in all skin biopsies, including affected and unaffected areas (Figs. 2–5). Abnormalities were most frequently observed in hair follicles (61% in biopsies with hair follicles), epidermis (52%) and sebaceous glands (45%), with less consistent effects in eccrine (sweat) glands (22%) (Figs. 2–5). Vellus hair growth appeared distorted or impaired (Figs. 3–5) with involution of anagen

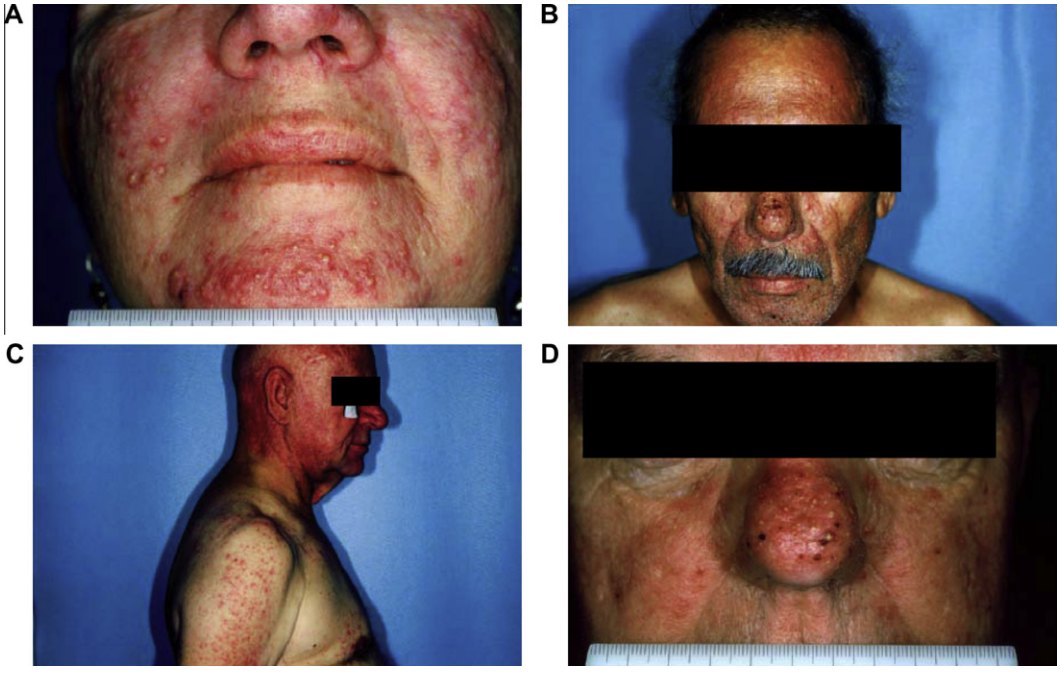

Fig. 1 – Erlotinib-induced rash is associated with diffuse telangiectasia (A) and occasionally serum crusts possibly indicating a secondary infection suggestive of impetigo (B and D). A representative example of the rash (C) shows extensive erythema and numerous, somewhat confluent, pustules over the entire head and extending to the neck and upper trunk.

Table 2 – Summary of skin biopsy pathology.

Tissue type	No. patients evaluable	Characteristic no. (%)
Epidermis		
Infiltrate	23	6 (26.1)
Neutrophil		6 (26.1)
Mononuclear		4 (17.4)
Eosinophil		0 (0.0)
Other		0 (0.0)
Any damage	23	12 (52.2)
Hair follicle		
Infiltrate	23	13 (56.5)
Neutrophil		10 (43.5)
Mononuclear		13 (56.5)
Eosinophil		1 (4.3)
Other		0 (0.0)
Any damage	23	14 (60.9)
Dermis		
Infiltrate	22	20 (90.9)
Neutrophil		9 (40.9)
Mononuclear		20 (90.9)
Eosinophil		2 (9.1)
Other		1 (4.5)
Any damage	22	1 (4.5)
Sebaceous gland		
Infiltrate	22	10 (45.5)
Neutrophil		5 (22.7)
Mononuclear		10 (45.5)
Eosinophil		0 (0.0)
Other		0 (0.0)
Any damage	22	10 (45.5)
Panniculus well represented		
Infiltrate	23	0 (0.0)
Neutrophil		0 (0.0)
Mononuclear		0 (0.0)
Eosinophil		0 (0.0)
Other		0 (0.0)
Any damage	23	0 (0.0)
Eccrine gland		
Infiltrate	22	5 (22.7)
Neutrophil		1 (4.5)
Mononuclear		5 (22.7)
Eosinophil		0 (0.0)
Other		0 (0.0)
Any damage	22	5 (22.7)
Vasculitis		
Present	23	0 (0.0)
Not present		23 (100.0)
Vasculitic haemorrhage		
Present	23	3 (13.0)
Not present		20 (87.0)
Epidermal thickness		
Normal	23	6 (26.1)
Abnormal		17 (73.9)
Thinned		1 (4.3)
Thickened		16 (69.6)

hair growth cycle (Fig. 4B) leading to keratin-plugged hair follicles (Fig. 2D), where abnormal numbers of remnant hair shafts from a single orifice were observed (Fig. 5D). Pilosebaceous glands (sebaceous glands associated with hair follicles) differentiating within the upper portion of the lumen were often very small, containing poorly differentiated sebocytes and keratinocytes (Figs. 3 and 5). Abnormal sebaceous glands had mild distortion of basal layer and less-ordered sebaceous differentiation than normal (Figs. 3C and D & 5B).

Abnormal epidermal thickening (acanthosis) was common in affected skin biopsies (74%) (Fig. 4B). Although epidermal thickness was not significantly reduced in 'unaffected' biopsies, altered terminal differentiation of corneocytes was observed (Fig. 4A). Whereas normal baseline stratum corneum had good granular layer and basket-weave appearance (Fig. 2A), this was not seen in 'unaffected' skin (Figs. 2E and 4A) and rash biopsies demonstrated reduced granular layer (Fig. 3C) and compact orthokeratosis (Fig. 4B), indicating aberrant terminal differentiation of corneocytes.

We detected vacuolisation of keratinocytes (dyskeratosis) in intra- or interfollicular spinous epidermis in both affected (Fig. 3B) and unaffected skin (Fig. 2E). The dyskeratosis in affected skin was associated with infiltration of mononuclear leucocytes into a miniaturised and distorted follicle (Fig. 3B).

Leucocytic infiltration was frequently observed in the dermis (91%), hair follicles (56%) and sebaceous glands (45%) (Fig. 4B–D). The infiltrate was mild to moderate in severity, located in superficial and deep dermis, both peri-vascular and around epidermal appendages (Fig. 5), and predominantly mononuclear in nature (dermis 91%, hair follicles 56% and sebaceous glands 45%). In areas with significant distortion of follicular structures or disruption of hair growth cycle, neutrophilic infiltration was observed in approximately 40% of cases (hair follicles 43%; dermis 41%). Bacterial colonies suggestive of potential *Staphylococcus aureus* infection (impetigo) (Fig. 5D), supported by clinical pictures showing honey-coloured crusts on various skin areas (Fig. 1B and D), were identified in only 6 (26%) of the 23 patients with TR and complete biopsies.

For further identification of mononuclear cells in the infiltrate, we analysed 10 representative samples of rash biopsies by immunohistochemistry for distribution of T-cells ($CD3^+$), Langerhans cells (LC) ($CD1a^+$), myeloid dermal DCs ($CD11c^+$), macrophages (CD163) and natural killer (NK) cells (CD56). Expression of inflammatory markers present on some DC subsets was also examined, including DC-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN), Tumour Necrosis Factor Related Apoptosis Inducing Ligand (TRAIL) and Toll like receptor 2 (TLR-2) (Fig. 6). Although LCs and $CD3^+$ T-cells were evident in the epidermis and dermis of the rash biopsies, respectively, they were not as abundant as the myeloid, $CD11^+$ DC population. The mononuclear infiltrate showed frequent TRAIL- and TLR-2-positive cells, with DC morphology. TRAIL, in particular, was highly expressed in rash biopsies. CD56 staining for NK cells was mostly negative (data not shown). Therefore, although the immune infiltrate of rash biopsies is partly composed of T-cells, LCs and macrophages, it is predominated by TRAIL-positive mononuclear cells with DC morphology (Fig. 6).

4. Discussion

The present study analyses the largest reported patient series treated with an EGFRI to specifically induce a typical

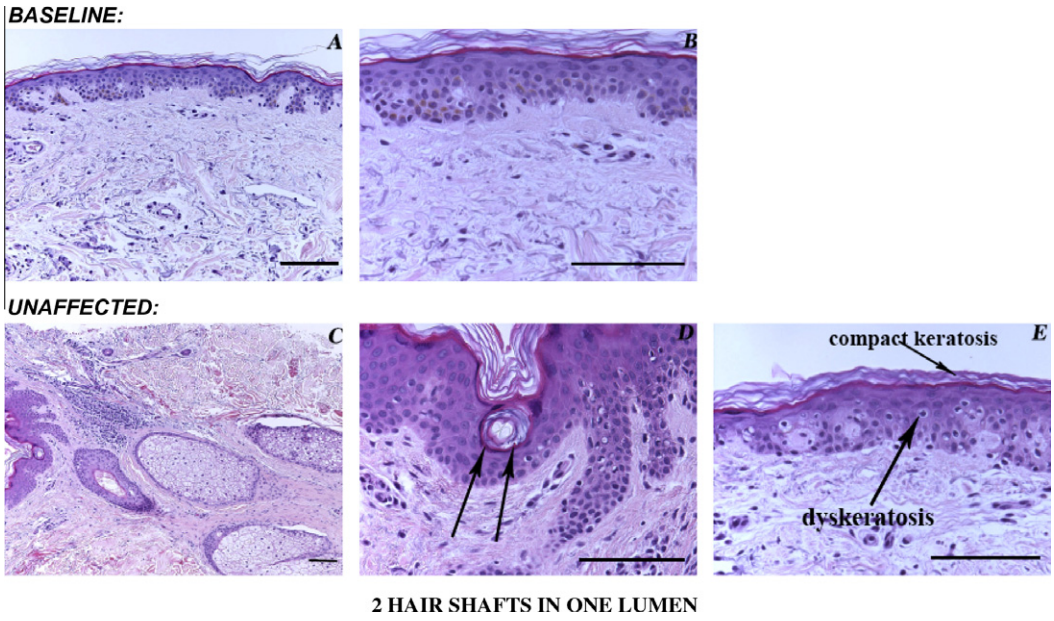

Fig. 2 – Altered corneocyte terminal differentiation and normal 'basket-weave' pattern of unaffected skin following treatment with erlotinib. (A and B) Baseline unaffected skin. Normal 'basket-weave' appearance and unaffected differentiation of stratum corneum. (C–E) Altered epidermal terminal differentiation of unaffected skin. Compact orthokeratosis, dyskeratosis (vacuolisation of keratinocytes) and disruption of hair follicle architecture, with several hair shafts in a single lumen following erlotinib treatment.

drug-associated rash and study its cellular basis. Unlike previous reports, the effects of EGFRI were studied in paired biopsies of 'affected' ('rash') and unaffected skin.

The 'acneiform' rash induced by EGFR inhibitors has been most commonly characterised as a suppurative folliculitis dominated by neutrophils within and around hair follicles that may also contain keratin-plugs and/or obvious bacterial colonies.[11,13] It is unclear from existing clinical studies whether neutrophil infiltration is a primary process, e.g. triggered by chemokines synthesised by keratinocytes or other epithelia in response to EGFR inhibition, or secondary to altered follicular structure and/or growth of bacteria within disrupted follicular structures.[31] Two mouse models of EGFR inhibition have been created to derive a better understanding of the clinical rash. In one model, a dysfunctional EGFR is engineered in skin epithelial cells.[22] The dominant effect in

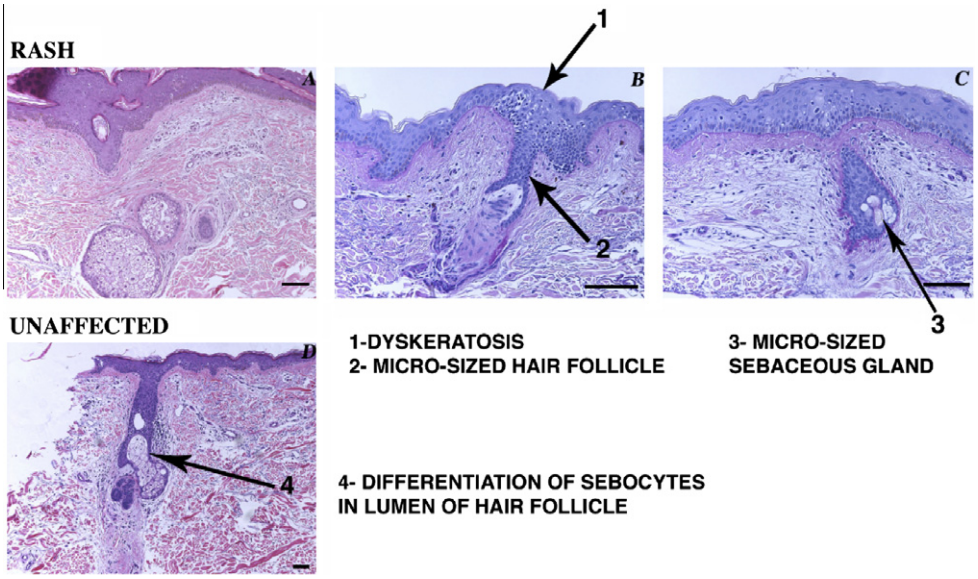

Fig. 3 – Disruption of pilosebaceous unit architecture in both rash (A–C) and unaffected (D) skin. Vellus hair growth was distorted with involution of anagen growth cycle leading to keratin-plugged follicles (B). Pilosebaceous glands were very small, displayed abnormal differentiation and contained poorly differentiated sebocytes (C and D).

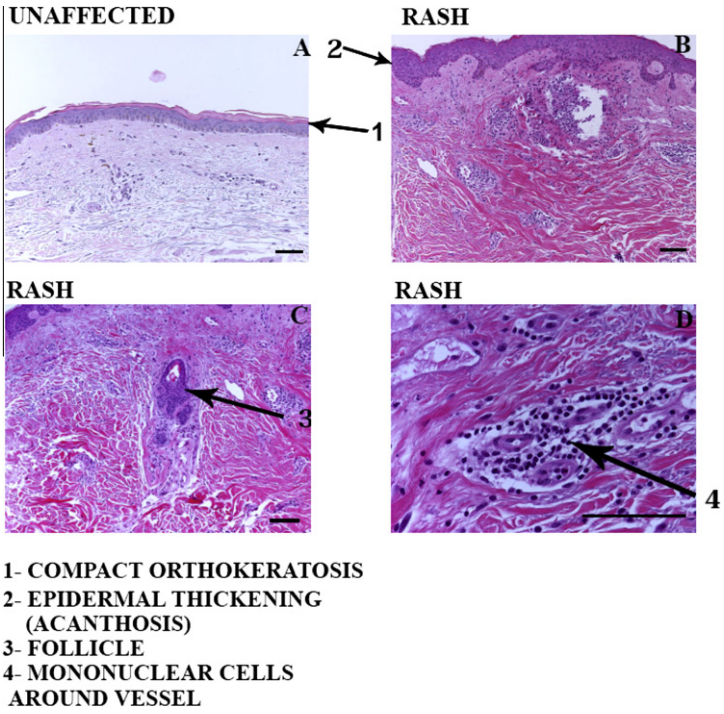

Fig. 4 – Aberrant terminal differentiation of unaffected (A) and rash (B) skin associated with a mononuclear infiltrate (C and D). Compact orthokeratosis and loss of 'basket-weave' appearance of unaffected and rash skin (A and B) with reduced granular layer and abnormal epidermal thickening only in rash skin (B). In affected skin a mononuclear infiltrate infiltrated the dermis and follicular structures (C and D).

this model is disruption of cyclic hair growth such that catagen (a phase where hair growth ceases) is not triggered and hair follicles become tremendously enlarged and pathologically invade deep muscle tissue. After several weeks, a secondary inflammatory infiltrate develops around degenerating deep-seated follicular structures. Since new hair growth is not triggered, a marked alopecia (hair loss) eventually becomes evident. Another EGFR inhibition model achieved with a monoclonal antibody leads to prevention of hair growth and marked hypertrophy of sebaceous glands that eventually triggers neutrophil influx (driven by increased TNF production from distorted follicles).[32] In both mouse systems, the primary pathology is disrupted growth of pilosebaceous structures with secondary induction of inflammation. Both models also display marked epidermal hyperplasia, which is largely unexpected from proposed EGFR biology in skin diseases.[31] In past clinical studies the effect of EGFR inhibition on unaffected skin was not investigated, so that the specific effects of EGFRI, that are independent of inflammation, could not be determined. Thus, it has been impossible to integrate clinical rash pathology with mechanisms and results obtained in model systems.

In the present study, we observed marked disruption of sebaceous gland growth (hypoplasia) and overall shrinkage of follicular structures in non-rash region (Fig. 3). These results show dominant effects on sebaceous glands and follicular structures that are independent of inflammation, in line with mouse models. However, the polarity of the response in human skin is different from mouse models, which are characterised by hypertrophy and not diminution of the pilosebaceous unit.[22,32] We also detected altered terminal differentiation of keratinocytes in affected and unaffected skin.

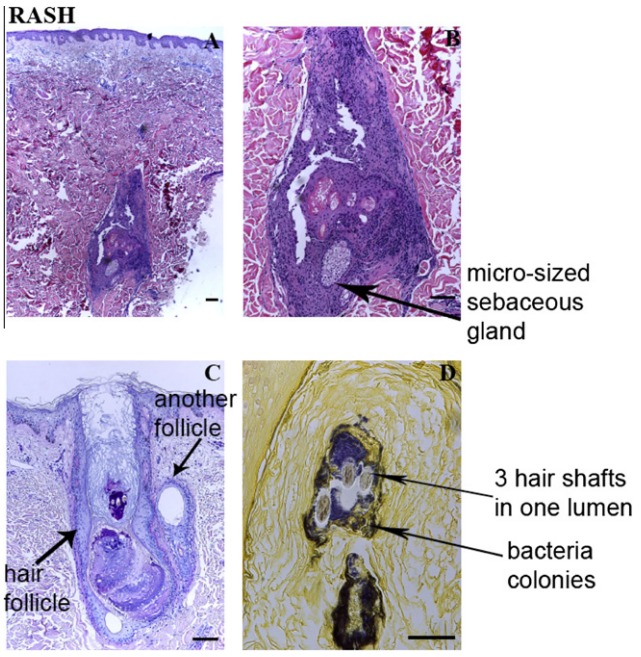

Fig. 5 – Bacterial colonisation of follicular structures associated with erlotinib-induced rash. Altered differentiation of sebaceous glands (A and B) and hair follicles, including several hair shafts in a single lumen (C and D). Bacterial colonies were detected in areas of follicular distortion (D).

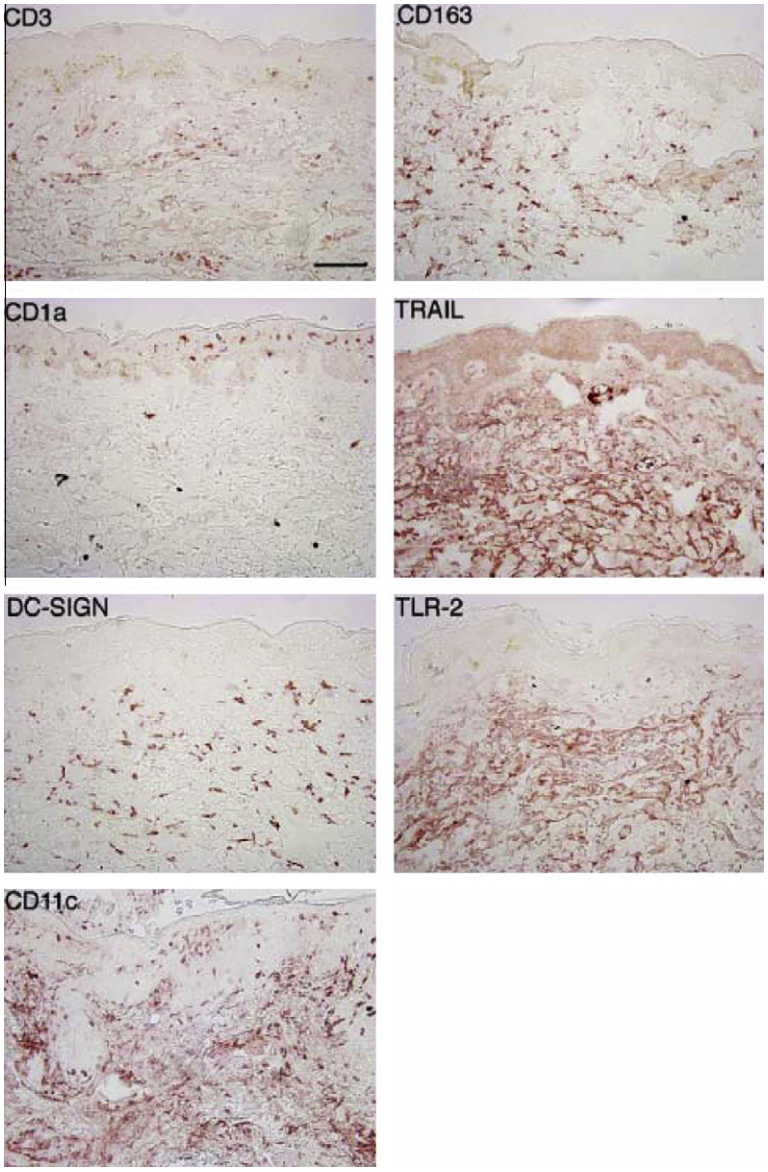

Fig. 6 – Characterisation of the mononuclear infiltrate by imunohistochemistry for markers of T-cells (CD3$^+$), dendritic cells (DCs) (DC-SIGN and CD11c$^+$), Langerhans cells (LCs) (CD1a$^+$), macrophages (CD163$^+$), natural killer cells (CD56$^+$) and inflammatory markers present on some DCs (TRAIL and TLR-2). Although the infiltrate was partly composed of T-cells, LCs and macrophages, it was predominated by TRAIL-positive cells with a DC morphology.

We observed a primary effect of EGFRI on growth/differentiation of entire pilosebaceous unit in both affected and unaffected skin, whereas the inflammatory changes could be detected in rash biopsies alone. Thus, we consider the primary process that triggers inflammation and eventual rash development to be related to altered growth/differentiation of follicular epithelium. Also, like the mouse models, we generally observed hyperplasia (increased thickness) of the epidermis in rash regions, in contrast to clinical reports on thinning of stratum corneum with EGFRI.[13] EGFR inhibition has been shown to worsen skin inflammation by increasing inflammatory mediator production.[31,33] Increased epidermal thickness in the setting of inflammation may result from the actions of inflammatory cytokines, thereby increasing keratinocyte proliferation.

Within rash regions, inflammation/leucocytic infiltration is peri-appendageal and generally associated with distortion or damage to the hair follicle-sebaceous unit. However, bacterial colonisation is also detected in a subset of rash biopsies. As proposed in mouse models,[22,32] inflammatory cell infiltration may well result from primary distortion/disruption of sebaceous gland and follicular structures. As rashes tend to develop in skin regions rich in sebaceous follicles, inflammation might be triggered by destruction of the relatively larger sebaceous glands in this type of follicles. Growth of bacteria within a distorted lumen of a follicle may be yet another trigger, especially for neutrophil infiltration. Unlike past studies, the composition of the infiltrating leucocytes is mainly mononuclear and not neutrophilic. Hence, the previous characterisation of the EGFRI rash as suppurative (neutrophilic)

folliculitis is inaccurate as it suggests a neutrophil-driven pathogenesis and fails to acknowledge the potential contribution of sebaceous gland pathology to the rash process. From immunohistochemical markers used, the mononuclear leucocytes are a mixture of T-lymphocytes ($CD3^+$), inflammatory dendritic cells ($CD11c^+$) and macrophages ($CD163^+$). Comedo formation, which is essential for acne vulgaris pathogenesis, is not observed in this study, in agreement with past work.[13]

Fig. 7A–C summarises the cellular and structural changes brought about by EGFR inhibition in human skin and contrasts pathogenic mechanisms with that of acne vulgaris. Overall, our findings have potential implications for treatment of the EGFR inhibitor rash and for the observed association between development of rash and therapeutic effect of EFGR inhibitors on underlying carcinomas.

Our studies also identify the TNF-family member TRAIL as a potential inflammatory mediator in rash regions. We demonstrate that the mononuclear infiltrate contains TRAIL-positive cells with DC morphology (Fig. 7C), which could be equivalent to TRAIL-positive inflammatory DCs identified in human[29] and mouse[28] cancers. TRAIL-positive inflammatory DCs have been shown to mediate apoptosis of cancer cells, by interacting with cell-surface death receptors DR4 (TRAIL-R1) and DR5 (TRAIL-R2), suggesting a role in cancer therapy.[28,34] Although mostly expressed by cancer cells, TRAIL receptors were demonstrated to be up-regulated on various epithelial cells, including keratinocytes[35] and prostate epithelial cells.[36] Evidence also exists for TRAIL involvement in inflammation in an NF-kappa B-dependent manner by inducing pro-inflammatory molecules (such as CCL20) that recruit effector cells, possibly increasing the

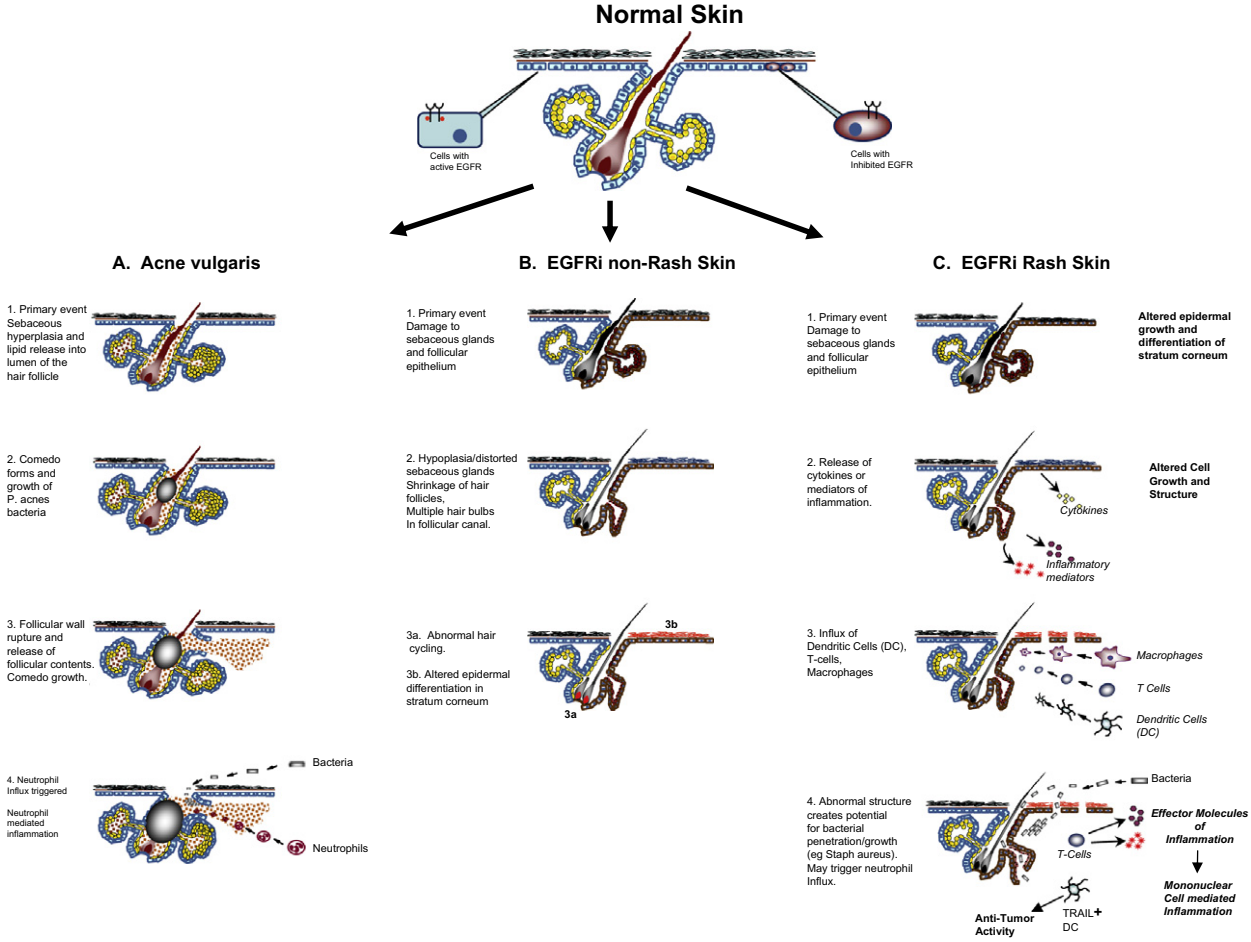

Fig. 7 – Skin toxicity model. There are important mechanistic differences between acne vulgaris (A) and EGFR toxicity in non-rash (B) and rash skin (C). This figure summarises the cellular and structural changes with EGFR inhibition (EGFRI) in affected and unaffected skin and contrasts pathogenic mechanisms with that of acne vulgaris. In acne (A), the primary process is sebaceous hyperplasia and lipid release into the follicular lumen leading to comedo formation and overgrowth of Propionibacterium acnes that results in follicular wall rupture, triggering neutrophil influx and pustule formation. In both unaffected (B) and affected (C) skin with EGFRI the primary event is damage to sebaceous glands and follicular epithelium, leading to altered epidermal growth and differentiation. In the EGFRI rash skin (C) damage to sebaceous glands or follicular epithelium triggers release of cytokines and immune-cell (mononuclear leucocytes) infiltration in a peri-appendageal pattern. This reaction is best classified as a 'sterile folliculitis', except in a minority of cases where distorted structures may have allowed bacterial entry.

tumouricidal effect of TRAIL.[37] TRAIL has been recently identified as a distinguishing marker of inflammatory DCs in human skin, that might drive pathology in various inflammatory conditions.[38] TRAIL thus might be a direct link between tumourigenicity and inflammation. Recently, an EGFRI, gefitinib (Iressa), was shown to be a potent enhancer of TRAIL-anti-cancer effects in human oesophageal squamous carcinoma.[39] Furthermore, a novel strategy that combines EGFR-signalling inhibition with target-cell-restricted apoptosis induction using a TRAIL fusion protein with specificity for EGFR was reported to have superior pro-apoptotic effects.[40]

The correlation between EGFRI drug activity and TR development and severity, together with a markedly increased expression of TRAIL-positive mononuclear cells in the dermis, suggests that TRAIL-positive DCs could directly contribute to rash formation. We hypothesise that erlotinib induces a TRAIL-positive inflammatory DC population and TRAIL interaction with death receptors on activated epithelial cells, or carcinoma cells, could lead to cellular apoptosis.

Since the changes identified in this study may reflect early erlotinib exposure, as TR biopsies were taken at first presentation, future studies should consider serial skin biopsies to investigate changes that occur from long-term exposure to an EGFRI. As TRAIL-induced apoptosis may be synergistic with EGFR inhibition in carcinomas[41] future studies should investigate the association of TRAIL-induced apoptosis in cancer tissue with TRAIL expression on immune infiltrating cells in both tumour and TR tissues.

The occurrence of skin infection in some patients may result from impaired skin barrier, perhaps stemming from disrupted hair follicle structure and/or altered differentiation of follicular epithelium (Fig. 7). EGFR inhibition was recently shown to reduce antimicrobial-mediated keratinocyte migration and proliferation,[31,41] possibly contributing to a disruptive skin barrier. Hence, a defective barrier combined with EGFR-driven reduction of innate immune mechanisms of the epidermis could contribute to development or severity of the skin rash.

The cutaneous pathology of EGFRI-induced skin toxicity described in this study suggests that many of the current therapeutic measures that are based on acne vulgaris treatment, in which the principal objective is to reduce comedo formation or growth of *Propionibacterium acnes*, may not be appropriate.[42,43] The rash does not typically respond to anti-acne agents and has been treated with anti-inflammatory, antibiotic and immunomodulatory agents such as corticosteroids, tetracyclines, pimecrolimus and tacrolimus. A better understanding of the mechanism of EGFRI-related skin toxicity may allow development of more specific therapeutics as well as optimising existing treatment modalities. Based on our results, the following treatment recommendations should be considered: (1) if skin lesions develop yellow crusts or show other signs of microbial infection, oral antibiotics that cover *S. aureus* or other demonstrated bacteria should be considered; (2) topical anti-inflammatory agents that suppress T-cell and dendritic cell activation may be helpful and (3) some inflammatory cells reside deep in the skin and may not be fully treated by topical agents due to limited penetration. Although systemic anti-inflammatory agents may be helpful for the rash, these agents could potentially interfere with immune-mediated anti-tumour actions and further work would need to be done to consider this option.

Conflict of interest statement

Authors Sean McCarthy and Kenneth Iwata are employed and own stock in OSI Pharmaceuticals, the sponsor of the phase 2 study from which biopsies were obtained for this characterisation study.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2010.04.028.

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