#### ABSTRACTS

#### P001 | First evidence of efficacy of an orally active RORγt inhibitor in the treatment of patients with moderate to severe plaque psoriasis

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RORyt is the master regulator of Th17 lymphocytes. Th17 cells are drivers of several autoimmune diseases including psoriasis. Using Vitae's proprietary drug design platform, potent and specific inhibitors of RORyt were designed, synthesized and assessed, and human studies initiated. Following SAD and MAD trials in normals, a double blind, placebo-controlled, proof of concept study was performed in 34 patients with moderate to severe psoriasis. VTP-43742 was administered orally once daily for 28 days. PASI scores and plasma IL-17A and F levels were determined at baseline and day 28, and % change from baseline calculated. In a subset of patients, IL-17A and F mRNA levels were determined in psoriatic lesions at baseline and day 28. Trough plasma drug levels were determined at weekly intervals. VTP-43742 was generally safe and well tolerated. Transaminase levels (ALT and AST) were elevated by greater than three times upper limit of normal in five out of 14 patients receiving the 700 mg dose with none in the 350 mg dose cohort. There were no SAEs, and one patient withdrew for mild nausea and facial flushing. VTP-43742 had a half-life of 2-3 days, requiring 2-3 weeks to reach steady state. PASI scores were decreased 21% and 29% from baseline at day 28 in the 350 and 700 mg/d cohorts, respectively. Plasma IL-17A levels were decreased at day 28 by 29% and 50%, respectively, in the 350 and 700 mg/d cohorts, and plasma IL-17F levels by 53% and 75%. Skin lesion biopsies after 28 days of dosing had a 63% and 66% decrease in IL-17F mRNA levels in the 350 and 700 mg/d cohorts, respectively, with no significant change in IL-17A mRNA levels. In summary, VTP-43742 is a once a day drug that was generally safe and well tolerated with promising evidence of efficacy. A larger, 16 week study is planned to increase the safety experience and to further assess efficacy.

#### P002 | Characterization of the chloroquineinduced pruritus mouse model and its pharmacological modulation using an automated platform

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Chronic Pruritus is a major symptom in many dermatological diseases as well as the manifestation of systemic disorders such as chronic kidney or cholestatic liver diseases. Pruritus has a dramatic impact on patients' quality of life and represents a large unmet medical need. Current treatments are based on first-generation sedative antihistamines and off-label use of analgesic and antidepressant drugs, which have limited efficacy and that most of the times exhibit CNS effects.

Chloroquine (CQ) is an antimalarial drug that induces generalized pruritus as a side effect in some patients. CQ has been used in mice to study the pathophysiology of histamine-independent itch. However, pharmacological validation is to date limited.

We have characterized the pruritus model in mice after CQ administration by different routes. Quantification of CQ tissue levels has been performed to monitor the site of action of the drug. Scratching count measurements have been performed in parallel to the measurement of other behaviour parameters such as locomotion, in an automated platform (laboras). Oral off-label used pruritus drugs and drugs in on-going clinical trials have been tested to assess whether this model is of translational value for the search of novel antipruritic agents.

Our results showed that all treatment options selected were able to inhibit pruritus induced by CQ, although in many of them the efficacy can be explained by central effects. Anti-histamines, antidepressants and k-receptor agonists inhibited scratching but, and at the same time, decreased locomotor activity. NK1 receptor antagonists were able to inhibit CQ induced itch without effects in locomotion.

Therefore, the acute CQ mice model, as it has been set up, has revealed a useful tool to understand the effect and limitations of currently used anti-pruritic drugs, as well as for the preclinical evaluation of novel mechanisms. The use of the automated platform allows to discriminate between real antipruritic activity and potential confounding factors such as sedation.

## P003 | The 5-year experience of the use of ustekinumab for psoriasis

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Ustekinumab is an anti-IL12/23 agent used for the treatment of psoriasis and psoriatic arthritis. Herein, we present our experience with the use of Ustekinumab in 93 psoriasis patients.

A chart review was conducted and 93 patients were identified, 50 men and 43 women. We recorded demographics, previous use of

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systemic and biologic drugs, history of psoriatic arthritis, safety and efficacy issues.

The mean age was 48.7 years and the mean duration of psoriasis was 18.6 years. 25.8% had a family history of psoriasis and 20.4% had psoriatic arthritis. 82.8% have already used Cyclosporine and 38.7% methotrexate. For 33.4% of the patients, Ustekinumab was the first biologic, 40.8% have used one biologic therapy, 22.6% have used two biologics and 3.2% had used 3. 47.3% were in the first year with Ustekinumab, 22.6% in the second, 13% in the third and 10.7% and 6.5% in the fourth and fifth. Mean PASI at week 0 was 10.28, at week 28 3.3 and at week 52 2.47.

Ten patients that also suffered from psoriatic arthritis, needed addition of methotrexate (doses from 7.5 to 12.5 mg/wk) with full remission of their symptoms, two patients needed addition of cyclosporine (due to pustular psoriasis) and one patient acitretin due to worsening of palmoplantar psoriasis. None of the above mentioned patients had to discontinue Ustekinumab. We had a patient who developed two BCC and a patient who developed one BCC and one SCC (this patient also suffered from albinism). One of our patients developed thyroid cancer (she has previously been treated with acitretin, methotrexate, adalimumab and UVBnb). In total we had to discontinue Ustekinumab in nine patients (4 because of loss of efficacy, 2 due to loss of public insurance, 2 for NMSC and 1 for thyroid cancer).

Ustekinumab was generally very well tolerated, has very good clinical results regarding its efficacy and based on our experience it also has a very good safety profile.

#### P004 | Clinical and histologic patterns of dermatologic toxicity from immune checkpoint blockade therapy

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**Background:** Immunotherapy that targets programmed cell death 1 (PD-1) receptor has demonstrated tremendous promise in the treatment of advanced solid tumors. Dermatologic toxicities, however, are an emerging consequence of this therapy. Nonspecific rash and pruritus may occur in up to 20% of patients treated with anti-PD-1 antibody therapy. The clinical and histologic patterns of dermatologic toxicity from immune checkpoint blockade are beginning to be recognized.

**Design:** Skin biopsies of dermatologic toxicity were reviewed from nine patients during the past 8 months. All clinical and histopathologic features were annotated.

**Results:** The clinical presentation included urticarial plaques, vesicles, and bullae with histologic and immunofluorescence features diagnostic of bullous pemphigoid (BP) in five patients (M:F=3:2; mean age, 70.2 years; range, 63-74) and violaceous papules, pustules, and plaques with histologic features diagnostic of lichenoid/interface dermatitis (LD) in four patients (M:F=3:1; mean age, 64.5 years; range, 59-74). Of

the BP dermatologic toxicity, anti-PD-1 antibody therapy (nivolumab: n=4; pembrolizumab: n=1) were for the following metastatic tumors: melanoma (n=1), head and neck squamous cell carcinoma (n=1), urothelial carcinoma (n=2), and pulmonary adenocarcinoma (n=1). Of the LD dermatologic toxicity, anti-PD-1 antibody therapy (nivolumab: n=3; pembrolizumab: n=2) were for the following metastatic tumors: melanoma (n=2) and lung adenocarcinoma (n=2). The mean onset of dermatologic toxicity after initiation of anti-PD-1 antibody therapy was 118 (range: 49-220) days for BP and 36.5 (range: 1-75) days for LD (P=.05). There was improvement of dermatologic toxicities with treatment of systemic and/or topical steroids. Patients with BP dermatologic toxicity required combined systemic and topical steroids while most patients with LD dermatologic toxicity were managed with topical steroids. All patients' immune checkpoint therapy was withheld. **Conclusion:** In our limited experience, the mean onset of BP dermatologic topical steroids.

tologic toxicity from anti-PD-1 antibody therapy appears days later compared with LD dermatologic toxicity. The awareness of specific patterns of dermatologic toxicity to immune checkpoint blockade therapy will become increasingly important for optimal patient care.

## P005 | Successful treatment of granuloma faciale with the addition of etanercept to methotrexate therapy

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Granuloma Faciale (GF) is a rare cutaneous disorder of unknown etiology that is notoriously difficult to treat. The differential for GF is broad and includes cutaneous T-cell lymphoma, sarcoidosis, erythema elevatum diutinum, discoid lupus erythematous, and fungal or microbial infections. Many therapies have been proposed to manage this difficult condition, but none have been very satisfactory. Recent therapies implemented to treat GF have included pulse-dyed laser, cryosurgery, topical tacrolimus, phototherapy, dermabrasion, oral dapsone, clofazimine, and topical, oral, and intralesional steroids, each with guarded success rates. In this case report, we present a 74 year-old male who initially presented with a ten-year history of an eruption that began on his nose and spread to his forehead, consistent with multiple lesions of granuloma faciale. Initial treatments included topical and intralesional corticosteroids as well as a short trial of hydroxychloroquine, followed by a trial of dapsone; after little improvement with any these therapies, cryotherapy was attempted and ultimately failed as well. Two and a half years later the lesions of GF remained, and the patient was empirically started on methotrexate. After only modest improvement, etanercept was added to the regimen on the premise that the inflammatory nature of his lesions would respond to a TNF-alpha blocking agent and it could

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add to the effects of the methotrexate. Two weeks after etanercept was started, the patient showed considerable improvement with most of the lesions appearing as brownish-red macules. Nearly a year after starting methotrexate therapy, the lesions have dramatically resolved to faded hyperpigmented macules. In summary, we report a patient who responded modestly to methotrexate therapy but dramatically so with the addition of a limited course of etanercept and has remained disease-free one year from initiation of treatment. We propose that the treatment of granuloma faciale, as well as other immune-mediated diseases of the skin, should be further investigated with these therapies.

### P006 | Free fatty acids sensitize dermal cells to amplify TH1/TH17-immune responses

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Obesity is associated with body fat gain and impaired glucose metabolism. Here, we identified both body fat gain in obesity and impaired glucose metabolism as two independent risk factors for increased serum free fatty acids (FFAs). Furthermore, obese subjects show increased and/or delayed resolution of inflammation observed in various chronic inflammatory diseases such as psoriasis. Thus, we hypothesized a possible impact of FFAs on immune cell function relevant for the pathogenesis of chronic inflammation. Surprisingly, stimulation of human monocyte-derived and mouse bone marrow-derived dendritic cells (DCs) with FFAs, such as palmitic acid (PA) and oleic acid (OA), did not affect the pro-inflammatory immune response of these immune cells. In contrast, pre-incubation with PA and OA sensitize DCs resulting in augmented secretion of TH1/TH17-instructive cytokines upon pro-inflammatory stimulation with LPS. Similar results according to a sensitization of dermal cells, such as keratinocytes and fibroblasts, to danger signals could be shown in this study by incubation with FFAs. The relevance of this observation was confirmed in vivo when obesity in mice worsened a TH1/TH17-driven psoriasis-like skin inflammation. A strong correlation of the amount of total FFA, PA and OA in serum and the severity of skin inflammation point to a critical role of FFAs in obesity mediating exacerbation of skin inflammation.

Our data suggest that increased levels of FFAs might be a predisposing factor promoting a TH1/TH17-mediated inflammation such as psoriasis in response to an inflammatory danger signal.

## P007 | Semaphorin 4D contributes to inflammation of psoriasis by inducing cytokine production

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**Background:** Psoriasis is an autoimmune skin disease, in which keratinocytes play a crucial role in the pathogenesis. Semaphorin 4D(Sema4D) is a pro-inflammatory factor that has been found expressed in keratinocyte membrane in psoriatic lesion. Whether Sema4D takes part in psoriasis development and the mechanism involved is not known.

**Objective:** To determine the proinflammatory and proliferative effect of Sema4D on keratinocytes and the contribution of Sema4D to psoriasis development.

**Methods:** We detected the expression of membrane Sema4D and its receptor Plexin-B2 in psoriatic skin lesion, moreover, the soluble and membrane Sema4D expression were detected in serum and T cells of PBMC from psoriasis patient. The HaCaT cells were treated by recombinant human soluble protein Sema4D and transfected with Plexin-B2 siRNA simultaneously, and then the expression of inflammatory factors, cytokines, chemokine and the intermediated signaling pathway were examined. Furthermore, the imiquimod-induced psoriasis-like Sema4D knockout mice and BALB/c mice were treated by Plexin-B2 siRNA fragment so as to investigate the proinflammatory role and Keratinocyte-T cells interaction of Plexin-B2/Sema4D in psoriasis development in vivo.

**Results:** The Sema4D was specifically expressed in psoriasis skin lesion and serum. A total of five psoriasis related inflammatory cytokines and chemokines were identified to be up-regulated by Sema4D stimulation in HaCaT cells. We then found that the activation of intracellular NF-κB,RhoA signaling pathway and NLRP3-Caspase-1 signaling pathway accounted for Sema4D-induced cytokines secretion. Moreover, applied Plexin-B2 siRNA fragment to BALB/c mice could contribute to alleviate the formation of psoriasiform dermatitis. Sema4D was released from lesional keratinocytes of imiquimod-induced psoriasislike Sema4D knockout mice and contributed to ameliorate the proinflammatory ability. In addition, the secretion of chemokines was inhibited by Plexin-B2 blockade.

**Conclusion:** Our findings indicate that secreted Sema4D from keratinocytes can facilitate the production of inflammatory cytokines and chemokines in keratinocytes, and contributed to alleviate the proinflammatory ability in Sema4D knockout mice. Blocking the proinflammatory function of Sema4D may be applied to psoriasis treatment in future.

## P008 | Expression of dysfunctional apoptosis regulating proteins bcl-2 and p53 in psoriasis vulgaris

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**Background:** Dysfunctional apoptosis has a very important role in the development of several inflammatory skin diseases. Psoriatic keratinocytes possess an enhanced ability to resist apoptosis, which might be one of the key pathogenetic mechanisms in psoriasis vulgaris. P53 and bcl-2 are two apoptosis controlling proteins. Studies have evaluated

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the expression of these two proteins in the psoriatic skin, but the results have been controversial.

**Methods:** Fifty-eight cases of psoriatic skin biopsies were studied, and the grade of bcl-2 and p53 immunostaining was correlated with the histopathological indices of severity.

**Results:** Bcl-2 expression in the epidermis strongly correlated with the expression in the basal cells and lymphocytes (P=.001 and .035). There was no correlation with epidermal hyperplasia or with p53 expression in the three compartments. Bcl-2 expression in the basal layer correlated with the p53 expression in the epidermis (P=.027), basal layer (P=.015) and the lymphocytes (P=.034). There was a strong correlation among the p53 expression in all the compartments. There was also a weak correlation of the p53 expression in the epidermis with the epidermal hyperplasia (P=.042).

**Conclusions:** Bcl-2 does not appear to play an important role in the apoptotic process in psoriasis. In contrast, it is likely that p53 has a far more important role to play. Mutation analysis of the p53 protein is necessary to evaluate if the protein has mutated or if it is of the wild type.

#### **P009** | Interleukin-1 gene polymorphisms and their relation with NFκB expression and histopathological features in psoriasis

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**Background:** Psoriasis is a chronic inflammatory disease driven by exaggerated production of pro-inflammatory cytokines and interleukins. Various genetic polymorphisms including IL-1 are implicated in pathogenesis of psoriasis. The exact role of IL-1 gene polymorphisms and their interaction with NF $\kappa$ B is not yet determined. We aimed to study various genetic polymorphisms of IL-1 in psoriasis and their influence on NF $\kappa$ B and histopathological features.

Materials and methods: 112 newly diagnosed cases of psoriasis vulgaris were included in this prospective study. Histology was done on sections and genotyping was done for the IL-1 $\beta$  and IL-1 receptor antagonist (IL-1RA) genetic polymorphisms. In addition, NF $\kappa$ B immunostaining was performed on 89 sections and the intensity of staining was evaluated in the epidermis, basal cells, and the lymphocytes.

**Results:** A strong association of IL-1 $\beta$  511 C/T polymorphism was found with both genotypes and alleles in psoriasis. A strong correlation was also detected between the IL-1 $\beta$  genotype and the grade of NF $\kappa$ B immunostaining in the epidermis (P=.012). The grade of NF $\kappa$ B lymphocyte staining showed a strong correlation with the IL-1RA genotype (P=.025) but not with the IL-1 $\beta$  genotype (P=.226). The genetic polymorphisms did not show any correlation with the histological features.

**Conclusions:** IL-1 genetic polymorphisms may not play a very direct role in pathogenesis of psoriasis. However, their interaction with NF $\kappa$ B appears to be a significant factor in this direction as NF $\kappa$ B is activated by pro-inflammatory genetic polymorphisms and therefore may influence the severity of psoriasis.

#### P010 | The pivotal role of wide local excision for the treatment of severe hidradenitis suppurativa (Hurley grade III): Retrospective analyses of 74 patients

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Hidradenitis suppurativa (HS) is a painful, chronic, recurrent inflammatory skin disease that affects terminal hair follicles and apocrine glands. It develops in early adolescence, and is confined to axillary and inguinogenital/gluteal regions. HS affects up to 4% of the general population. This study aimed to measure the impact of surgery on the individual quality of life in severe grade HS patients. Additionally, parameters such as disease duration, previous therapeutic interventions, postoperative complications (pain, infection, scarring/keloids, wound healing deficiency, mobility restrictions), postoperative recurrence and satisfaction with the cosmetic results were evaluated. Data from 74 patients (40 male, 34 female) with HS Hurley grad III treated with wide local excision and secondary wound healing were evaluated. Most patients had inguinogenital/gluteal disease (n=51, 68.9%, P<.001). Inguinogenital/gluteal disease was pronounced in female patients (P=.009). Involvement of both, axillary and inguinogenital/gluteal areas were pronounced in male patients (P=.018). Most patients (n=53; 71.6%) had a disease history of more than 5 years at the time of initial presentation at our institution. Wide local excision improved the Dermatology Life Quality Index (DLQI) scores from initially 27.89 (range 2-30; SD=5.3) to 5.31 (range 0-26; SD 7.38; P<.001) independent of localization (P=.195). 47.3% of patients had postoperative complications, most frequently pain and scarring. Local recurrence rates were calculated with 18.9% from follow-up data covering a period of up to 14 years. 70.3% of patients were highly satisfied with the cosmetic results. From our study we conclude that wide local excision of affected skin significantly improves the quality of life of HS Hurley grad III patients and has low rates of local recurrence. Further, surgery has the potential to locally heal HS areas. Satisfaction with the cosmetic results is high. The socio-economic footprint of wide local excision, to date, favors surgery over systemic therapies with anti-inflammatory biologics.

#### P011 | Etanercept, adalimumab and ustekinumab in psoriasis vulgaris: detailed retrospective analysis of 209 treatment series in 134 patients in a single institution in Austria

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**Background:** Biologic agents are widely used in psoriasis vulgaris patients and have been tested in many clinical trials. Drug efficacies

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and adverse events may differ in 'real world` patients who are not selected and monitored as rigorously.

**Objective:** To report drug survival, efficacy, and adverse events (quality, time of onset) in real world psoriasis vulgaris patients treated with etanercept, adalimumab and ustekinumab.

**Methods:** Retrospective data analysis (Jan-01-2004 to Jun-30-2015) in a psoriasis clinic in an Austrian tertiary referral hospital. All patients (209 treatment series in 134 patients) who received at least one dose of etanercept, adalimumab or ustekinumab for the treatment of psoriasis vulgaris and had at least one follow up visit were included in the analysis. We analyzed: patient demographics, drug survival, Psoriasis Area and Severity Index (PASI), quality and time of onset of adverse events.

**Results:** In 209 treatment series in psoriasis vulgaris patients the estimated median drug survival differed between treatments: 21 months (SE: 6.9) for etanercept, 61 months (SE: 9.4) for adalimumab and 65 (SE 1.4) for ustekinumab. Male gender and pre-treatment with a biologic agent were positive predictors for longer drug survival in adalimumab; no such predictors were identified for etanercept and ustekinumab. We found no significant difference in drug efficacy measured by PASI.

**Conclusion:** We confirm the safety of etanercept, adalimumab and ustekinumab in patients with psoriasis vulgaris and show that most AEs happen during the first year of treatment. Adalimumab and Ustekinumab have higher drug survival times compared to etanercept.

#### P012 | Improvement of DC vaccination strategies: Identification of a stable and migratory subset of IL-10 modulated dendritic cells

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The immunosuppressive cytokine IL-10 induces tolerogenic dendritic cells (IL-10DC) which serve as potent regulators of immunity due to their ability to induce anergic regulatory CD4+ T cells (iTregs). Within IL-10DC we identified two subpopulations: CD83highCCR7highHLA-DRhigh and CD83lowCCR7negativeHLA-DRlow L-10DC. Compared to mature DC, CD83low IL-10DC showed diminished expression of costimulatory molecules and slight upregulation of inhibitory molecules like ILT3 and ILT4. In contrast CD83high IL-10DC revealed minor alterations in the expression of costimulatory molecules but showed increased expression of inhibitory molecules. However both subsets of IL-10DC irrespective of their grade of maturation were potent inducers of iTreg which suppressed the activation of responder T cells. We aimed to analyse in detail the different suppressive capacities of iTreg induced by the two subsets of IL-10DC and found that that regulatory CD4+ T cells (iTreg+) induced by CD83high IL-10DC

exhibited a significantly higher suppressive capacity compared to CD4+ regulatory T cells (iTreg-) generated by CD83low IL-10DC. Furthermore iTreg+ revealed a higher degree of activation by means of proliferation and cytokine secretion when compared to iTreg-. With the perspective in mind to use tolerogenic DC for iTreg induction in clinical settings a potent migratory phenotype of DC as well as stability under inflammatory stimulation are crucial. We found that CD83high rather than CD83low IL-10DC exerted a stronger migratory capacity towards the lymph node-related chemokine CCl21 and exhibit a stable phenotype under inflammatory conditions. Furthermore, CD83high express high levels of surface and soluble CD25 (sCD25). Functional analysis revealed that sCD25 secreted by tolerogenic IL-10 DC did prevent T cell activation which could counterbalance the DC-mediated tolerance induction. Prospectively the selective use of CD83high IL-10DC for tolerance induction in vivo may contribute to an improvement of DC vaccination strategies against allergic and autoimmune diseases.

#### P013 | Location is everything: RNA-seq of distinct anatomic regions in psoriasis reveals distinct transcriptomic signatures for scalp, palmoplantar, and conventional plaque psoriasis

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Clinicians have long recognized that anatomic location is an important feature for defining distinct subtypes of plaque psoriasis. However, little is known about the transcriptomic differences between scalp psoriasis, palmoplantar psoriasis, and conventional plaque psoriasis which typically presents on the trunk and extremities. To investigate the molecular heterogeneity that may exist between scalp, palmoplantar, and conventional plaque psoriasis, we performed RNA-seq on 4 mm punch biopsy samples from four individuals with conventional plaque psoriasis, five individuals with scalp psoriasis, two individuals with palmoplantar psoriasis, along with nine samples from healthy control patients. We performed differential expression analysis using CuffDiff and performed network analysis using weighted gene coexpression network analysis (WGCNA). Differential expression analysis revealed 1483 DE genes between psoriasis of the scalp and conventional psoriasis, 648 DE genes between palmoplantar psoriasis and conventional psoriasis, and 1378 DE genes between scalp psoriasis and palmoplantar psoriasis. WGCNA revealed scalp and palmoplantar psoriasis specific coexpression modules (Pearson p>.7, P<5x10-4). This work reveals the molecular heterogeneity of plaque psoriasis and identifies subtype-specific signaling pathways that will aid in the development of therapy that is appropriate for each subtype of plaque psoriasis.

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#### P014 | In situ mapping of innate lymphoid cells in healthy and inflamed human skin

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Innate lymphoid cells (ILCs) have recently been identified as potentially important players of cutaneous immune homeostasis. In this study, we aimed at developing a technique to assess ILCs in situ and to determine their topographical distribution in human skin.

We collected lesional skin biopsies from atopic dermatitis (AD) and psoriasis (Pso) patients (both n=13) and normal human skin (NHS) from healthy controls. We established immunofluorescence ILC in situ staining panels and then developed an analysis approach (gating combined with manual validation) to reliably identify ILCs. Topographical mapping was obtained by automated calculation algorithms of the distances between ILCs and different cellular/structural elements of the skin.

Whereas a very scarce ILC population (mostly ILC1s and AHR+ILC3s) was detected in NHS, AD and Pso skin lesions harbored clearly increased numbers of ILCs. We observed AD skin to contain not only ILC2s, but also a prominent AHR+ILC3 population. Almost equal proportions of ILC1s and RORC+ILC3s were found in Pso skin. Distance calculations revealed ILCs to reside near the epidermis but not blood vessels. Strikingly, all ILC subsets showed a close proximity to T lymphocytes.

In conclusion, in situ mapping of ILCs will provide valuable information about the likely communication partners of these cells in normal and diseased skin and forms the basis for the appropriate mechanistic studies.

#### P015 | Transient neonatal zinc deficiency caused by a novel mutation in SLC30A2 gene

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Background: Transient neonatal zinc deficiency (TNZD) has a clinical presentation similar to that of acrodermatitis enteropathica, and is caused by defective mammary gland secretion of zinc into milk.

Aim: To present a case of TNZD in an exclusively breastfed infant. Materials and methods: This is a case report of a four-month old full-term, fully-breastfed Chinese baby who presented with persistent peri-orificial and groin rash associated with poor weight gain and irritability. The infant's serum zinc level was low. The mother's breast milk zinc level was also low, whereas her serum zinc levels were normal, confirming the diagnosis of transient neonatal zinc deficiency. Mutational analysis revealed a novel mutation in the mother's SLC30A2 gene, which encodes a zinc transporter expressed in mammary gland epithelial cells.

Conclusion: Breast milk zinc levels should be analysed and TNZD considered a differential diagnosis when an exclusively breastfed infant presents with zinc deficiency.

#### P016 | De novo missense variant in the KIT proto-oncogene in a child with piebaldism

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Piebaldism is a rare congenital pigmentation disorder caused by the absence of melanocytes and melanin in certain areas of the skin. It is characterised by depigmented patches of skin and hair.

We report the findings in a Eurasian girl with mixed Chinese and German ancestry. She was born prematurely at 33 weeks of gestation via emergency caesarean section for IVF dichorionic diamniotic twins in labour. She has an unaffected non-identical twin sister. There was no family history of pigmentation disorders. At birth, she was noted to have a white forelock with patches of depigmentation and multiple café-au-lait macules on her limbs and trunk. Her cognitive and physical development is normal for her age.

Genomic DNA was extracted from venous blood and sequenced using the TruSight One panel on the MiSeq System. Genetic sequencing revealed a heterozygous missense mutation in KIT gene. This variant results in the substitution of Leucine with Arginine in the intracellular tyrosine kinase domain of the KIT protein. It has not been reported in the Human Gene Mutation Database or in published literature. It is not found in the DNA from the saliva sample of her unaffected sister or the blood samples of her parents. This change is predicted to be pathogenic by SIFT and Polyphen2.

The phenotype of the patient is consistent with the diagnosis of piebaldism with depigmented patches and islands of normal/hyperpigmented skin. The presence of a pathogenic variant in the heterozygous state is consistent with the molecular genetics of this autosomal dominant disorder. Our report of this novel missense variant adds to the spectrum of pathogenic mutations in the KIT gene in patients with piebaldism.

#### P017 | IgE sensitization profiles differ between adult patients with severe and moderate atopic dermatitis

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**Background:** Atopic dermatitis (AD) is a complex chronic inflammatory disease where allergens can act as specific triggering factors.

**Aim:** To characterize the specificities of IgE-reactivity in patients with AD to a broad panel of exogenous allergens including microbial and human antigens.

Methodology: Adult patients with AD were grouped according to the SCORAD index, into severe (n=53) and moderate AD (n=126). As controls 43 patients were included with seborrhoeic eczema and 97 individuals without history of allergy or skin diseases. Specific IgE reactivity was assessed in plasma using Phadiatop<sup>®</sup>, ImmunoCap<sup>™</sup>, micro-arrayed allergens, dot-blotted recombinant Malassezia sympodialis allergens, and immune-blotted microbial and human proteins.

**Results:** IgE reactivity was detected in 92% of patients with severe and 83% of patients with moderate AD. Sensitization to cat allergens occurred most frequently, followed by sensitization to birch pollen, grass pollen, and to the skin commensal yeast M. sympodialis. Patients with severe AD showed a significantly higher frequency of IgE reactivity to allergens like cat (rFel d 1) and house dust mite (rDer p 4 and 10), to Staphylococcus aureus, M. sympodialis, and to human antigens. In contrast, there were no significant differences in the frequencies of IgE reactivity to the grass pollen allergens rPhI p 1, 2, 5b, and 6 between the two AD groups. Furthermore the IgE reactivity profile of patients with severe AD was more spread towards several different allergen molecules as compared to patients with moderate AD.

**Conclusion:** We have revealed a hitherto unknown difference regarding the molecular sensitization profile in patients with severe and moderate AD. Molecular profiling towards allergen components may provide a basis for future investigations aiming to explore the environmental, genetic and epigenetic factors which could be responsible for the different appearance and severity of disease phenotypes in AD.

## P018 | Application of peanut protein on normal skin leads to tolerance whereas application on irritated skin leads to IgE sensitization

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**Background:** In epicutaneous immunotherapy (EPIT), allergens are applied on intact skin, which leads to desensitization in humans and animal models. The aim of this study was to compare the immunological response induced by EPIT on intact or inflamed (stripped) skin in sensitized mice and to identify the mechanisms involved in these disparate responses.

**Methods:** BALB/c mice sensitized to peanut protein were treated by EPIT using a Viaskin peanut patch for 8 weeks applied either on normal intact or on irritated skin. A major peanut allergen, Ara h 1, was measured in plasma samples by ELISA. Following EPIT, mice received an oral peanut regimen, aimed at triggering an allergic reaction. Mucosal samples were taken for histology and cytokines mRNA measurement. Plasma specific antibodies were also measured. In parallel, mechanisms of antigen capture in the intact and irritated-skin were investigated in OVA-sensitized mice by analyzing the phenotype of migrating dendritic cells (DCs) and lymph node cell responses.

**Results:** Ara h 1 was detectable in plasma of mice treated on irritated skin. Following EPIT on intact skin, slgE decreased and slgG2a increased whereas following EPIT on irritated-skin, slgE increased and slgG2a was not modified. Esophageal eosinophil infiltration was greater in Sham and irritated-skin EPIT than in EPIT and naive. Eotaxin, IL-5, IL-13 and GATA-3 mRNA expressions were significantly reduced by EPIT compared to Sham and irritated-skin EPIT-treated mice. Moreover, after applying Viaskin to intact skin, migrating DCs expressed a high level of MHC-II, CD205, CD86, CD83 and PIR-B and were positive for intracellular IL-12 (iIL12). On irritated skin, by contrast, application of Viaskin<sup>®</sup> migrating DCs expressed lower levels of CD205, MHC-II, PIR-B and no iIL12.

**Conclusions:** EPIT on intact normal skin delivers allergen to the superficial epithelium, which induces specific activation and maturation of migrating DCs, leading to an effective desensitization process. On irritated skin, this therapeutic effect is dramatically reversed.

## P019 | Complementary in vitro activity of two compounds on non-histaminergic pathway of pruritus

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Pruritus is one of the most common skin complaints, not always with a skin disease associated. Pruritus could hit every age group but its prevalence is high in elderly patients. Itch constitutive of pruritus can trigger sensitisation by histaminergic and non-histaminergic pathways. H1/H4 Histamine receptors stimulation, the histaminergic classical pathway will not be addressed in this study. Indeed, epidermal PAR-2 receptors (Protease-activated receptor-2) activation mediates cutaneous itch directly, not suppressed by antihistamine compound with a specific neuronal stimulation pathway (Davidson et al., 2010). TRPV1 channel (Transient receptor potential vanilloid 1) can be activated at the skin level by heat, pH and osmolarity modulation, physicochemical effects present during itch. Furthermore, its expression is increased in elderly patients epidermis (Lee YM et al., 2008).

The aim of this work is to evaluate active ingredients to target PAR-2 and TRPV1 activation pathways to inhibit pruritus induction. The use of keratinocytes cell line HaCat allowed us to assess PAR-2 activity after stimulation by trypsin or SLIGKV-NH2 peptide agonist in monitoring Ca2+ flux signals. In this model, Polidocanol showed a significant dose response inhibitory activity after trypsin stimulation

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and a competitive activity against peptide agonist at the highest concentration.

Prucidine-4<sup>®</sup> efficacy was tested on TRPV1 recombinant CHO cell based assay. Ca2+ flux signals modulation was measured after capsaïcin agonist stimulation. Prucidine-4<sup>®</sup> showed a significant dose response inhibition with a full reverse effect.

These data suggest that a topical Dermo-Cosmetic product combining Polidocanol and Prucidine-4<sup>®</sup> compounds for their PAR-2 and TRPV1 inhibitory effects respectively could be useful to protect against pruritus induction.

#### P020 | Identification of an expression-based set of genes to predict the subset of patients with systemic scleroderma (SSc)

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**Introduction:** SSc is a heterogeneous connective tissue disease. We have previously shown that based on clinical characteristics, SSc-overlap patients clearly differ from the diffuse (dcSSc) and the limited form (lcSSc). To confirm the clinical diversity, we have used expression profiling in whole blood to differentiate patients from healthy controls (HC) and to identify a specific gene-set for classifying patients into the main SSc-subsets.

**Materials and methods:** Four centers collected blood of 150 patients and 40 HC. Total RNA was extracted, gene expression analysis, Student's *t* test and fold change analysis was performed. Subsequently, a molecular predictor was created by pairwise comparison of the subtypes. This gene-set was used as a training-set for three support vector machines (SVMs). The predictive potential of these SVMs was evaluated by a leave-one-out cross validation.

**Results:** The analysis revealed 43 differentially expressed genes between all SSc patients and HC, of which 40 were interferon regulated. This was also true for those genes which were overlapping, but additionally, specific, non-overlapping genes clearly differentiate all subsets from each other.

The SVM-based predictions enabled us to distinguish patients with dcSSc/lcSSc with a success rate of 94.2%, dcSSc/SSc-overlap syndromes with a 81.7% and lcSSc/SSc-overlap syndromes with 77.3% success rate. Most of these underlying predictive genes were functionally associated with cellular growth/proliferation, cell signaling, cell death/survival and clinically associated with inflammatory and infectious diseases.

**Discussion:** These data clearly support the clinical hypothesis that lcSSc, dcSSc and SSc-overlap syndromes represent separate subsets within the spectrum of scleroderma with specific genes for different subsets. We have also identified a gene-set which allows the classification of patients into clinical entities without making use of existing clinical traits, and, importantly, without skin biopsies as the data was derived from peripheral blood. In the future, this will enable us to identify subsets of patients at an early stage of the disease, predict the course of the disease in individual patients better and to decide about potential therapeutic options.

### P021 | Association between atopic dermatitis and autoimmune disease in adults

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**Background and objective:** An increased susceptibility to autoimmune disease has been shown in patients with atopic dermatitis (AD), but data remain scarce and inconsistent. The aim of this study was to examine the co-occurrence of selected autoimmune diseases in adult patients with AD.

**Materials and methods:** Nationwide health registers were utilized. Adult patients with a hospital diagnosis of AD in Denmark between 1997 and 2012 were included as cases (n=8112), and matched with controls (n=40,560). The occurrence of autoimmune diseases was compared in the two groups. Logistic regression was used to estimate odds ratios. Multivariable analyses were adjusted for age, sex, socioeconomic status, smoking and an index for health utilization.

**Results:** AD was significantly associated with alopecia areata (aOR 26.31; 95% CI, 14.48-47.80), vitiligo (aOR 17.98; 95% CI, 7.70-42.01), chronic urticaria (aOR 9.92; 95% CI, 6.43-15.32), celiac disease (aOR 5.19; 95% CI, 2.93-9.20), chronic glomerulonephritis (aOR 4.17; 95% CI, 2.16-8.07), Sjögren's syndrome (aOR 3.74; 95% CI, 2.41-5.82), systemic lupus erythematosus (aOR 2.65; 95% CI, 1.63-4.31), ankylosing spondylitis (aOR 2.33; 95% CI, 1.42-3.83), Crohn's disease (aOR 2.09; 95% CI, 1.52-2.85), unspecified IBD (aOR 2.07; 95% CI, 1.33-3.24), ulcerative colitis (aOR 1.64; 95% CI, 1.31-2.05), rheumatoid arthritis (aOR 1.61; 95% CI, 1.29-2.01). No significant associations were found between AD and autoimmune thyroid diseases, multiple sclerosis, type 1 diabetes, autoimmune hematological diseases or pulmonary fibrosis. AD was furthermore associated with having multiple autoimmune comorbidities. Patients with a history of smoking had a significantly higher occurrence of autoimmune comorbidities compared to non-smokers.

**Conclusion:** The results suggest a susceptibility of autoimmune diseases in adult patients with AD, especially in smokers. Furthermore, the results support the notion that Th1 and Th2 dominated diseases are not mutually exclusive. Increased awareness of autoimmune comorbidities in patients with AD may be warranted and smoking should be discouraged.

## P022 | Prevalence of comorbidity and associated risk factors in adults with atopic dermatitis and psoriasis

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**Importance:** Atopic dermatitis (AD) is a common chronic skin disorder, which may persist into adulthood, however the prevalence of comorbidities in patients with AD is not well characterized. AD is considered a systemic disorder like psoriasis, which has raised a need for data on the comorbidity profile of patients with AD, to assess the potential risks, benefits, and complications in management of patients with AD. **Objectives:** To describe the occurrence of medical and psychiatric comorbidities and associated risk factors in adults with AD compared with psoriasis and the general population.

**Participants:** All Danish individuals aged ≥18 years with a hospital (inpatient or ambulatory) diagnosis of AD or psoriasis during the study period (January 1st 1995 to December 31st 2012) were linked in nationwide registers.

**Outcomes and methods:** We examined the prevalence and risk of comorbidity and associated risk factors (smoking, alcohol abuse, hypertension, myocardial infarction, stroke, cancer, renal disease, diabetes, Crohn's disease, ulcerative colitis, depression, antidepressant use, anxiety, anxiolytic use, and statin use) using logistic regression.

**Results:** Overall, the prevalence of smoking and alcohol abuse was higher among AD patients compared with the general population, but lower compared with patients with psoriasis. Similarly, patients with AD generally had more risk factors and a higher prevalence of comorbidity than general population controls, but a lower prevalence and reduced risk compared to patients with psoriasis, except for use of anxiolytics, which was higher in AD patients with severe disease. The prevalence of diabetes was lower in AD patients when compared to patients with psoriasis as well as general population controls.

**Conclusion and relevance:** Despite an increased risk of various medical and psychiatric comorbidities compared to general population controls, adult patients with AD had a much lower prevalence of cardiovascular disease than patients with psoriasis. However, the prevalence of psychiatric co-morbidity and tobacco smoking was alarmingly high in severe AD patients, which might be target for intervention in management of patients with AD.

## P023 | Local cyclic nucleotide repression inhibits Melanoma growth

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Increases in nucleotide signaling molecules suppress innate and adaptive immune cell function and contribute to melanoma growth. Here we report that cyclic adenosine monophosphate (cAMP) accumulates in growing melanoma. Repression of intratumoral cAMP formation by a nucleotide cyclase inhibitor or (Inducible cAMP early repressor, ICER)-deficiency impaired melanoma growth but did not affect a cAMP-poor control tumor. Nucleotide cyclase inhibition initiated substantial changes in the tumor microenvironment, including a reduction of the regulatory T cell (Treg) and neutrophil infiltrate, reversal of T cell exhaustion and relocation of IFN- $\gamma$ -producing T cells from the tumor site to the draining lymph node. Analyzing the phenotype and function of tumor-infiltrating monocytes upon second messenger repression and in lineage-specific ICER-knockouts revealed a key role of tumor-infiltrating monocytes in cAMP-mediated melanoma immune suppression. When combined with partial Treg depletion, pharmacological cAMP repression achieved complete remission of established tumors.

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To summarize, our study presents a novel strategy for metabolic reprogramming to increase anti-tumor immunity and bolster immunotherapy.

#### **P024** | Vδ1+T-cells are stress-sentinels in human skin and are implicated in alopecia areata pathogenesis

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γδ T-cells regulate hair follicle (HF) cycling and execute stress surveillance tasks in murine skin. Yet, their role in human skin physiology remains unknown. Therefore, after confirming the presence of V $\delta$ 1+T-cells in/around human scalp HFs, we investigated how Vδ1+NKG2D+CD69- dermal γδ T-cells isolated from human scalp skin, impact on human HFs ex vivo. Despite their autologous nature, co-cultured dermal Võ1+T-cells became activated and exhibited marked HF cytotoxicity against "stressed", but not against "nonstressed" scalp HFs ex vivo (comparable to autologous CD8+T-cells). These  $\gamma\delta$  T-cells induced HF dystrophy, premature catagen, HF overexpression of the stress-induced NKG2D ligand, MICA, and HF immune privilege (IP) collapse. Most of these phenomena were inhibited by co-administering blocking antibodies against INFy, CD1d, or MICA. Thus, human dermal Vδ1+T-cells likely recognize "stressed" HFs via CD1d-presented HF self-antigens and MICA binding to NKG2D+ followed by INFy secretion. This promotes HF dystrophy, HF IP collapse and premature catagen entry. Since this chain of events strikingly mimics alopecia areata (AA) pathogenesis, we also investigated the role of  $\gamma\delta$  T-cells in AA. Indeed, dense infiltrates of NKG2D- and INFγ-overexpressing Vδ1+T-cells were detected in/around lesional HFs from AA patients and V $\delta$ 1+T-cells were present around affected HFs in experimentally induced AA lesions in human scalp xenotransplanted onto SCID mice. These data suggest that dermal V $\delta$ 1+T-cells -WILEY—Experimental Dermatology

in human skin serve physiological functions as stress-sensing sentinels, which attain pathological significance in AA. Thus, targeting excessive activities of NKG2D+V $\delta$ 1+T-cells may be a promising, novel adjunct therapy in AA and related autoimmune diseases.

#### P025 | Blistering of lesional skin is a sensitive and specific method to measure markers of disease activity in patients with vitiligo

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Vitiligo is a common skin disease that results from melanocyte destruction mediated by cytotoxic-CD8+ T cells. Existing treatments are non-targeted, and provide only modest efficacy. We previously found that IFN- $\gamma$ -induced CXCL9 and CXCL10 are critical for vitiligo pathogenesis, and hypothesized that inhibiting the IFN- $\gamma$ -chemokine axis could be an effective targeted treatment strategy.

Future trials to test the efficacy of new treatments will require early markers of treatment responses because clinical responses are slow. We, and others, reported that serum levels of CXCL9 and CXCL10 are elevated in disease, and correlate with disease activity as well as treatment responses. However, serum biomarkers are insensitive and nonspecific because vitiligo presents with minimal inflammation that is confined to the epidermis, and other diseases also increase serum chemokine levels. Measuring disease markers directly in the skin should improve their sensitivity and specificity, however tissue analysis by histology and flow cytometry is limited by selective sectioning and destructive processing. Here we report a novel method to directly sample cells and proteins from superficial skin fluid through suction blistering. This approach revealed an increased number of CD8+ T cells within vitiligo lesions compared to non-lesional and healthy control skin (P=.0078). Many of these T cells were tetramer-positive, recognizing melanocyte antigens. The ROC analysis indicated the test was 83% sensitive and 61% specific in identifying active vitiligo lesions (AUC=.8021, P=.0006), while CXCL9 protein was elevated, with a sensitivity and specificity of 70% and 97% (AUC=0.868, P<.0001).

Taken together, these data indicate that skin CD8+ T cell number and CXCL9 are sensitive and specific markers of disease activity in vitiligo patients. Superficial sampling of interstitial skin fluid through suction blisters could be a convenient, non-scarring, and minimally invasive method to monitor disease activity and treatment responses in future studies.

### P026 | Skin tissue engineering: Human healthy and disease skin models

<u>S. Gibbs</u> VU University Medical Centre, Amsterdam, Netherlands All skin disease has an underlying immune or genetic component. Due to differences between animal and human physiology, the majority of drugs fail in the pre-clinical and clinical testing phase. Therefore animal alternative methods that incorporate human immunology and genetic disorders into in vitro skin disease models are required to move the field forward. This lecture will focus on our tissue engineered organotypic skin models and their potential use in drug discovery and testing.

When designing a skin equivalent model it is most important to only introduce the level of complexity which is required for the particular research question. Simple models may incorporate a reconstructed differentiated epidermis with or without a fibroblast populated matrix. Complexity may be introduced by adding for example endothelial cells or adipose derived stem cells to the dermal matrix or melanocytes into the epidermis. Immune cells can also be incorporated eg Langerhans Cells in the epidermis and dermal dendritic cells in the dermis. By constructing the skin models with cells isolated from diseased tissue biopsies or cell lines, disease models can be created eg keloid, melanoma. However, even our most advanced models are still inadequate for many of our research questions since they lack the two way migration of immune cells into and out of the skin via the blood and lymph vessels and they lack the interaction with other organs in the body eg the lymph node, liver, kidneys. It is possible that the next generation of human skin disease models using skin-on-a-chip technology may provide some of the solutions.

#### P027 | Increased relapses and complications in pemphigus patients treated by the same physicians in a public safety net versus a private University Healthcare System

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Access to healthcare and its relationship with socioeconomic status has been documented for different diseases. Information on how different healthcare systems, which vary widely in terms of incentives for doctors, affect the care of patients with complex diseases is lacking.

The aim of this study was to determine whether any patient care disparities existed when patients with pemphigus, a severe mucocutaneous autoimmune blistering disease requiring on-going immunosuppression, are treated by the same physicians in two different healthcare systems, ie, a county-funded safety net hospital system (Safety Net System) and a private university hospital system (Private System).

We performed a retrospective chart review study of 65 patients with pemphigus vulgaris and foliaceus who were managed in the Safety Net System (n=34) and Private System (n=31) between July 2001 and May 2015.

Patients in the two systems did not differ considerably with regards to applied treatments and achievement of clinical or immunological remission. Safety Net System patients, however, experienced more disease relapses with a shorter recurrence-free survival time after achieving remission and more infectious adverse events. The greater rate of medication non-compliance observed in the Safety Net System patients likely played a role in creating these disparities. Other potential contributory factors are ethnicity, language, and/or socioeconomic status.

#### P028 | Improvement of anti-inflammatory and antibacterial effects of combined crude drug extracts including barafu on atopic dermatitis

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**Background:** Long-term use of steroid as a cure for atopic dermatitis can cause side effects. Therefore, there has been research on commercialized use of natural combined crude drug extracts as medicines recently. Against this backdrop, the study aims to investigate the clinical, anti-inflammatory and anti-bacterial effects of combined crude drug extracts including Barafu on atopic dermatitis patients.

**Methods:** The combined crude drug extracts used for the study include various plant bodies such as Barafu, Phellinus linteus (SANG HWANG Mushroom) and formulated into a cream product. Analysis of anti-inflammatory effect was conducted using cell toxicity evaluation by MTT assay, and gene expression related to anti-inflammatory was measured with primers of TNF (tumor necrosis factor)- $\alpha$ , COX (cyclooxygenase)-2 and IL (interleukin)-1 $\beta$ . Also, anti-bacterial activity was measured by conducting antibacterial activity for staphylococcus aureus. Clinical effectivity evaluation was conducted through SCORing Atopic Dermatitis (SCORAD) index and patients' global assessment of clinical response, and itching sensation was assessed with 10 cm-visual analogue score.

**Results:** As a result of analysis on the anti-inflammatory effect, Ethanol (EtOH) solvent extract had little effect on the expression of inflammation genes, while Ethyl acetate (EA) and Methylene chloride (MC) solvent extracts showed decreased expression of TNF- $\alpha$ , COX-2 and IL-1 $\beta$ gene, showing anti-inflammatory effect. As for anti-bacterial effect, combined plant extract containing Barafu showed improved anti-bacterial activity compared to other natural single plant extracts. Also, the clinical symptom evaluation showed significant decrease in objective SCORAD index before and after treatment.

**Conclusion:** Based on the results, it was revealed that combined plant extracts and the application products including Barafu had anti-inflammatory and anti-bacterial effects and improvement of itchiness for atopic dermatitis patients. Therefore, follow-up studies on mois-turizing effect and clinical stability for patients are expected to be contributable for atopic dermatitis medicines.

#### P029 | Topical application of WOL074-019 tripeptide exhibits anti-inflammatory activity in murine model of psoriasis

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KdPT, a tripeptide with broad anti-inflammatory activity, was shown to be effective in different murine models of intestinal inflammation and psoriasis. Unfortunately, KdPT's physicochemical properties are not favorable for development of a topical formulation. Thus, we designed and synthesized analogues of KdPT with optimized physicochemical properties. The anti-inflammatory activity of WOL074-019 (19) was comparable to KdPT as determined in vitro and was thus selected for in vivo studies.

The anti-inflammatory and immunomodulatory potential of substance 19 in vivo was first investigated after intravenous (i.v.) application in the mouse model of imiquimod-induced psoriasis-like skin inflammation. At days 5 and 6 after the start of imiguimod treatment mice were injected with either PBS, betamethasone dipropionate (BDMP), KdPT or 19 (all 5 µg, i.v.). Treatment with 19 ameliorated ongoing skin inflammation as shown by the reduced epidermal thickness, decreased levels of Th1 and Th17 cells in regional lymph nodes and lesional skin as well as the down-regulated levels of pro-inflammatory cytokines like IL-1β, IL-6 or TNF-α. Effects of 19 were comparable to the effects of KdPT and BDMP. Next, we wanted to determine whether local application of 19 might be sufficient to ameliorate ongoing imiquimodinduced psoriasis. The same imiquimod-induced murine model was topically treated at d5, 6 and 7 after the start of imiquimod application with either vehicle cream or cream with 1% emulsified substance 19. Notably, mice locally treated with 19 showed a significant amelioration of skin inflammation, as demonstrated by the comparable reduction in epidermal thickness or decrease of effector cell activation. Interestingly, topical treatment was as efficient as i.v. treatment.

In summary, these data show that 19, similar to the original tripeptide KdPT, is able to effectively ameliorate ongoing inflammation in skin. Because of its improved physicochemical properties 19 can be formulated as topical drug where it shows similar efficacy like systemically applied KdPT or BDMP. Moreover, following topical application its activity was found to be superior to KdPT.

#### P030 | Interest of I-modulia, aquaphilus dolomiae extract, in immune inflammatory response of atopic dermatitis pathology

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Atopic dermatitis (AD) is a chronic inflammatory skin disease with a complex pathophysiology, including genetic, immunologic and -WILEY—Experimental Dermatology

environmental factors interactions, and characterized by allergic inflammation, barrier disruption and pruritic lesions. Keratinocytes which are active immunological cells with major control over AD inflammation by means of cytokine/chemokine production contribute to the disease phenotype development. Likewise, TH1, TH2, TH17 cells which are commonly observed in AD lesions are postulated in the disease process. Emollient application makes the use of topical potent immune modulatory agents logical therapeutic consideration. The aim of the present study was to evaluate I-modulia, an original biological extract from culture of Aguaphilus dolomiae, isolated from Avene Spring Thermal Water microflora, on different immune inflammatory cell models. Firstly, inflammatory mediators gene expression was assessed in Normal Human Keratinocytes (NHK) stimulated by the association polyI:C+IL4+IL13+TNFα and performed by Q-PCR 32 genes array. Secondly PAR-2 (Protease-activated receptor-2) activity was assessed in NHK stimulated by trypsin and performed by Ca2+ flux signals monitoring. Finally TH1, TH2, TH17 cytokines basal production was assessed on CD4+ lymphocytes by 12-plex Luminex technology. Our results showed that I-modulia inhibited gene expression of numerous mediators including TSLP, IL18, IFN<sub>β</sub>1, RANTES, MCP3, TARC, MIP-3a, MDC, IL4R, and induced involucrin, psoriasin, RNase7 gene expression; I-modulia also inhibited the PAR-2 activation and the TH1, TH2, TH17 cytokines production (IL2,IFNy/ IL4,IL5,IL13/ IL17, respectively). Together, the present data support the high regulator activity of I-modulia, an Aquaphilus dolomiae extract, on keratinocyte inflammatory and lymphocyte immune responses, and reveal its powerful interest in topical preparations designed for the regulation of AD immune inflammatory pathology.

## P031 | Interest of I-modulia, an Aquaphilus dolomiae extract, in innate immune response of atopic dermatitis pathology

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Atopic dermatitis (AD) is a highly chronic relapsing inflammatory skin disease characterized by skin barrier dysfunction occurring through both genetic and acquired mechanisms, and associated to immune response alteration. The innate defective immune response in AD patients has been shown to enhance susceptibility to skin infection, in particular by Staphylococcus aureus; moreover impaired innate mechanisms such as pattern recognition receptors (PRRs) and antimicrobial peptides (AMPs) lead to impaired recognition of microbial components. A polymorphism in the Toll-Like Receptor-2 (TLR-2) gene with AD has been reported and linked to these functional impairments. As well, the production of AMPs has been reported to be impaired in AD. In this way, the aim of this study was to evaluate an original biological extract named I-modulia from culture of Aquaphilus dolomiae, isolated from Avene Spring Thermal Water microflora, on TLRs activation and on AMPs expression. Activation of TLR-1 to

TLR-10 by I-modulia was assessed on recombinant HEK-293 cell lines which functionally over express each TLR protein and SEAP gene reporter. Expression of AMPs was assessed on normal human keratinocytes incubated with I-modulia before mRNA quantification by Quantigene technology. Our results showed that I-modulia activated TLR-2, TLR-4 and TLR-5. Moreover, I-modulia induced, like flagellin, a TLR-5 natural ligand, mRNA expression of three AMPs: hBD-2, cathelicidin and psoriasin. These inductions were blocked by TLR-5 blocking antibody. Together, the present data support the notable activity of I-modulia, an Aquaphilus dolomiae extract, on innate immunity by TLR-2, TLR-4, TLR-5 activation, and by AMPs induction via mainly TLR-5 activation. This TLRs activation is highly interesting especially as strategies that boost TLR-2 are now also reported to restore epidermal integrity in AD, besides the restoration of innate immune response.

#### P032 | MicroRNA-146a suppresses IL-17-mediated skin inflammation and is genetically associated with psoriasis

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Psoriasis is an immune-mediated inflammatory skin disease with dysregulated interaction between keratinocytes and immune cells, where activation of IL-17 signaling is central in the pathogenesis. Little has been known about the role of non-coding RNAs, including microR-NAs, in the predisposition to psoriasis.

The genetic association of the functional polymorphism within the microRNA-146a (miR-146a) precursor (rs2910164) to psoriasis was assessed on a Swedish cohort of 1546 psoriasis patients and 1526 controls. A strong protective association of rs2910164 with psoriasis, in particular with early-onset disease, was found. Using the imiquimodinduced mouse model of psoriasis, we demonstrate that mice genetically deficient in miR-146a display earlier onset and exacerbated pathology of skin inflammation, with increased skin thickness and erythema, epidermal hyperproliferation, increased neutrophil infiltration as well as increased expression of IL-17-induced keratinocyte-derived inflammatory mediators. Strikingly, miR-146a-deficient mice fail to resolve inflammation after discontinuation of imiquimod-challenge. Functionally, miR-146a regulates the sensitivity of keratinocytes to IL-17: its overexpression suppresses while its inhibition enhances IL-17-induced inflammatory responses. Moreover, miR-146a impairs the neutrophil chemoattractive capacity of keratinocytes. Finally, delivery of miR-146a mimics into skin alleviates psoriasiform skin inflammation, with suppression of IL-17-signaling, decreased epidermal proliferation and neutrophil infiltration.

Our results highlight the role of microRNAs in psoriasis susceptibility and define a crucial role for miR-146a in the restoration of skin homeostasis after an inflammatory challenge by suppressing IL-17driven inflammation.

#### P033 | KPI-150, a potent inhibitor of Kv1.3 potassium channels demonstrates preclinical proof of-concept efficacy against atopic dermatitis when applied topically

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KPI-150 is a potent and specific peptide inhibitor of Kv1.3 potassium channels on effector memory T cells (TEM). TEMs of both CD4 and CD8 lineages are key drivers of autoimmunity and are pathogenic in multiple autoimmune diseases. TEM's utilize Kv1.3 voltage-gated potassium channels for sustained intracellular calcium influx, proliferation and activation. Immunofluorescence and immunohistochemistry analyses demonstrate that atopic dermatitis (AD) skin lesions from moderate-to-severe AD patients show high levels of Kv1.3, and that most of the infiltrating T-cells in the AD lesions express Kv1.3, validating the target relevance for KPI-150 in AD. Topical application of KPI-150 was evaluated in an oxazolone elicited AD model in rats with ear thickness measured on day one and two post challenge. Administration of KPI-150 shows dose-dependent reduction in inflammation. T-cell infiltration, and ear thickness in this model. These results along with previous work with a related peptide, dalazatide, which demonstrated proof of concept efficacy in a Phase 1b psoriasis clinical trial, demonstrate that KPI-150 has potential therapeutic benefit in AD, and other dermatologic inflammatory diseases when administered topically.

#### P034 | A 0.05% hypochlorous acid gel displays anti-itch and anti-inflammatory properties in a chronic mouse model of atopic dermatitis: Possible direct effect on neurons

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It has been reported that a topical hypochlorous acid formulation leads to relief in itch in human atopic dermatitis patients, however, the exact mechanism of action remains unclear. Thus we tested a 0.05% hydrogel formulation in a chronic mouse model of house dust miteinduced itch and inflammation as well as in vitro effects of hypochlorous acid on dorsal root ganglia (DRG) sensory neurons.

NC/Nga mice were sensitized with house dust mite (HDM) allergen twice weekly and treated topically twice a day with hypochlorous acid in a preventive as well as in a therapeutic setting. Endpoints were lesional skin score, scratching behavior and post-mortem cytokine profile in treated skin as well as total IgE in serum.

The topical administration of hypochlorous acid prevented the development of any lesions and the scratching bouts were significantly reduced during the whole observation period of 12 weeks. When administered after fully development of lesions, hypochlorous acid reduced lesions and scratching behavior to the same extent as a 0.1% betamethasone dipropionate ointment. Reduction of lesions was confirmed histologically and by reduced concentration of atopy-related cytokines/chemokines, like IL-4 and TARC in HDMchallenged skin. In addition, total serum IgE levels were significantly reduced by hypochlorous acid treatment. The reduced scratching behavior was confirmed by reduced concentrations of pruritus associated cytokines like IL-31 and TSLP in HDM-challenged skin and a reduced response rate to several pruritogens in DRG sensory neurons excised from NC/Nga mice after termination of the studies. Interestingly, the response to the stimuli was also reduced by pre-incubation of DRG sensory neurons from untreated BALB/c mice with 0.0001% hypochlorous acid.

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Taken together, these data indicate an anti-inflammatory and antiitch potential of hypochlorous acid, the latter possibly due to a direct reduction of sensory response.

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#### P035 | UV-induced inhibition of adipokine production in subcutaneous fat aggravates dermal matrix degradation in human skin

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Ultraviolet (UV) exposure to the human skin reduces triglycerides contents and lipid synthesis in the subcutaneous (SC) fat. Because adiponectin and leptin are the most abundant adipokines from the SC fat, we aim to investigate how they interact with UV exposure and skin aging. The expressions of adiponectin and leptin were significantly decreased in SC fat of sun-exposed forearm skin, in comparison with that of sun-protected buttock skin of the same elderly individuals, indicating that chronic UV exposure decreases both adipokines. Acute UV irradiation also decreased the expressions of adiponectin and leptin in SC fat. The expressions of adiponectin receptor 1/2 and leptin receptor were significantly decreased in the dermis as well as in SC fat. Moreover, while exogenous adiponectin and leptin administration prevented UV- and TNF-a induced matrix metalloproteinase (MMP)-1 expression, they also increased UV- and TNF- $\alpha$  induced reduction of type 1 procollagen production. Silencing of adiponectin, leptin or their receptors led to an increased MMP-1 and a decreased type 1 procollagen expression, which was reversed by treatment with recombinant human adiponectin or leptin. In conclusion, UV exposure decreases the expression of adiponectin and leptin, leading to the exacerbation of photoaging by stimulating MMP-1 expression and inhibiting procollagen synthesis.

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#### P036 | Hidradenitis suppurativa is associated with familial Mediterranean fever: A populationbased study

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Background: Hidradenitis suppurativa (HS) is a progressive inflammatory skin disease with an unclear pathogenesis. Familial Mediterranean fever (FMF) is the most common monogenic autoinflammatory disease. Our clinical experience has included several patients in whom HS and FMF occurred concurrently. This is in line with a very recently published small case series of patients diagnosed with both diseases. Objective: To examine a possible association between HS and FMF.

Methods: A cross-sectional study was conducted in a large, community-based cohort of patients with HS and age- and gendermatched controls. Patients were considered positive for HS when diagnosed by a dermatologist. All patients with FMF in the cohort and the control group were identified, and their files were manually reviewed to validate co-diagnoses of FMF and HS or diagnosis of FMF without HS respectively; based on clinic visits, surgical procedures, and pharmacy claims. The proportion of patients with FMF with and without HS was compared. The association between HS and FMF was assessed using a multivariate logistic regression model, adjusting for age, sex, ethnicity, socioeconomic status, obesity, and smoking.

Results: The study included 4417 patients with HS and 22085 controls. FMF was diagnosed in 33 patients with HS (0.7%) and 15 controls (0.1%) (P<.001). On univariate analysis, HS was significantly associated with FMF [odds ratio (OR) 11.1, 95% confidence interval (CI) 6.0-20.4]. This significant association was maintained on multivariate analysis [OR 6.6, 95% CI 3.2-13.4].

Conclusions: HS is strongly associated with the autoinflammatory disease, FMF. This association, together with reports on patients with HS showing good response to IL-1 alpha receptor antagonist therapy, supports the theory that HS is an autoinflammatory disease. Our findings highlight new potential avenues to follow in deciphering the pathogenesis of HS.

#### P037 | Investigation of the effect of tofacitinib on keratinocytes

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Tofacitinib is a novel, small-molecule Janus kinase (JAK) inhibitor, which has demonstrated efficacy in the treatment of psoriasis in

clinical trials. Tofacitinib inhibits JAK1 and JAK3 and to a lesser extent JAK2. The mode of action by which tofacitinib exerts efficacy in treating psoriasis is not completely understood but it has been thought to be mediated by the inhibition of CD4+ T-cell activation. In this study, we investigated whether tofacitinib can modulate the activity of the JAK/STAT-pathway in keratinocytes, which has been strongly linked to the development of psoriasis lesions. Primary human keratinocytes were treated with tofacitinib and then stimulated with IL-22. a key effector cytokine in psoriasis which signals through JAK1/Tyk2. Transcriptomic profiling revealed that IL-22 has a significant impact on the transcriptome both at 6 hours and 24 hours. More than 90% of the identified IL-22-regulated transcriptomic changes were prevented by pretreatment with tofacitinib, demonstrating that tofacitinib has a profound effect on the keratinocyte transcriptome and IL-22-induced signalling. Interestingly, tofacitinib altered even the baseline expression of genes in the absence of IL-22-stimulation in keratinocytes. Pathway analysis revealed significant enrichment of genes in the JAK/ STAT-pathway among the genes regulated by tofacitinib. Gene ontology analyses revealed pathways related to keratinocyte differentiation among tofacitinib-regulated transcripts in keratinocytes. qPCR analyses confirmed the upregulation of S100A7 and downregulation of EGR1 expression by IL-22, which was prevented by tofacitinib pretreatment. These results indicate that oral and topical treatment with tofacitinib may exert its effects in psoriasis partly via the modulation of IL-22-induced keratinocyte gene signatures.

#### P038 | Role of fucosvltransferase 8 in pathogenesis of epidermal proliferation/ differentiation and psoriasis development

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Psoriasis is a major skin inflammation disease characterized by excessive growth and aberrant differentiation of keratinocytes. Triggers to such responses by keratinocyte have been shown to involve activations of the cellular immune system, T cells, dendritic cells and various immune-related cytokines and chemokines. In recent years, different genes and proteins have been highlighted as potential biomarkers for psoriasis, but most of these are not psoriasis specific. Nevertheless, the pathogenesis of psoriasis remains poorly understood, and the effectiveness of disease treatments still leaves much to be desired. We investigated the role of mammalian  $\alpha$ 1, 6-fucosyltransferase (FUT8) in psoriasis epidermal development in human samples, animal models, and human keratinocyte cell-line. FUT8 catalyzes the transfer of a fucose residue from a donor substrate, GDP-fucose, to the Nlinked acetylglucosamine of the core structure complex glycopeptides. Core-fucosylation, or a1,6-fucosylation, plays an essential role in various pathophysiological events, such as cell growth. We found that in both human and animal skin samples, FUT8 and its core-fucosylated glycoproteins were correlated with skin epidermis proliferation and the formation of psoriasis phenotypes. Using skin epidermis from

human samples and mass spectrometry methodologies, we identified various  $\alpha$ 1,6-core-fucose glycoproteins, which were involved in the regulation of keratinocyte proliferation and differentiation. We constructed FUT8 knockdown/overexpressed clones to investigate the mediating function of FUT8 in the dimerization of epidermal growth factor receptor (EGFR), a cell surface receptor-linked tyrosin kinase for extracellular ligands. The binding affinity between ligands and EGFR was also found to be affected by FUT8. We further illustrated the downstream EGFR signaling pathways, such as PI3K/Akt, MAPK/ERK and NF- $\kappa$ B, which were significantly regulated by FUT8 in human keratinocyte. PI3K/Akt and MAPK/ERK are involved in cell proliferation and differentiation, whereas NF- $\kappa$ B is a key modulator of immunity and inflammation. These findings revealed the complex regulatory mechanisms and dynamic functions of EGFR in keratinocyte, which was heavily modulated by core-fucosylation.

#### P039 | Th2 cells and basophils expressing IL-4 together with ILC2 expressing IL-13 infiltrate skin lesions of patients with atopic dermatitis but not psoriasis

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**Background:** Atopic dermatitis (AD) is a common chronic skin inflammatory disorder orchestrated by type 2 immune response. Initiation and perpetuation of AD involve interactions between innate and adaptive type 2 cells that include basophils, innate lymphoid type 2 cells (ILC2) and Th2 cells. Novel promising therapeutic approaches for moderate-to-severe AD include a humanized antibody directed to IL-4Rα chain, shared by IL-13R. Here, we investigated the frequency of type 2 cells and the source of IL-4 and IL-13 in skin and blood of patients with AD when compared to psoriasis (Pso).

**Methods:** Blood and lesional skin biopsies were collected from AD and Pso patients. Skin cell suspensions were prepared by combining mild enzymatic digestion and mechanical dissociation. IL-4 and IL-13 expression were analyzed at the single-cell level with or without prior in vitro culture and stimulation using flow cytometry.

**Results:** (1) The frequency of Th2 and ILC2 was significantly higher in skin and blood of AD relative to Pso patients. Conversely, Th17 and ILC3 predominated in Pso. (2) Basophils were detected in skin of AD but not Pso. (3) Skin Th2 cells that expressed IL-4 prior to in vitro stimulation were detected in AD but not Pso while skin IL-13 expression was observed in Th2 cells in the two diseases. (4) In vitro stimulation induced similar IL-4 and IL-13 production in blood and skin Th2 cells from AD and Pso. (5) In vitro stimulated-basophils produce IL-4 but not IL-13. Conversely, ILC2 produce IL-13 but no IL-4.

**Conclusions:** (1) Tissue infiltrate is a better reflection than blood profile to assess the cellular source of cytokines in these two common skin inflammatory diseases. (2) Lesional AD, but not Pso skin, is infiltrated by Th2 cells as well as basophils which secrete IL-4 and, ILC2 which are IL-13-producers. Thus, innate and adaptive type 2

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cells differentially contribute to the production of pro-inflammatory cytokines observed in the skin of patients with AD.

#### P040 | Expression level of microRNA in the peripheral blood and tissue of untreated and narrowband ultraviolet B (NBUVB) treated active generalized vitiligo

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Vitiligo is an acquired autoimmune depigmenting disorder characterized by loss of functional melanocytes from the epidermis leading to patchy loss of pigmentation. There are growing evidences suggesting the role of microRNA (miRNA) in the pathogenesis of autoimmune diseases. The NBUVB is one of the effective therapies used in the treatment of generalized vitiligo leading to successful repigmentation. However, the understanding of involvement of miRNA in vitiligo etiopathology is limited and the modulating effect of NBUVB on miRNA expression is not known in vitiligo. The present study investigated the expression level of 10 microRNA (miRNA) in the peripheral blood and lesional and non-lesional skin of untreated and NBUVB treated active generalized vitiligo (aGV) patients (n=15) and healthy individuals (n=15). Expression levels of miR145 and miR16 were found to be significantly increased (P<0.05) in the peripheral blood, and were also increased in lesional compared to non-lesional skin of aGV. No significant correlation found between expression level of miR145 and miR16 and percentage body surface area involvement of patients, however, a significant positive correlation was found for miR203 (p=0.529; P=0.023). The expression of miR145 and miR16 were significantly decreased (P<0.05) in peripheral blood after NBUVB treatment compared to untreated aGV. Further, the expression of miR145 and miR16 were decreased in both lesional and non-lesional skin after NBUVB treatment. The above mentioned observation suggested the involvement of miR145 and miR16 in the pathogenesis of aGV and the study is first to report that the NBUVB treatment may induce pigmentation by modulating the expression of miRNA. However, further investigation is in progress to explore the mechanistic regulation of melanogenesis by miRNA in vitiligo and may lead to identification of novel miRNA that can be targeted for the management of vitiligo.

### P041 | CD109 is a key regulator of the IL-23/IL-17 immune axis in the skin

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A dominant response following cutaneous infection is production of interleukin (IL)-23 from dendritic cells that drives innate cell WILEY-

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production of IL-17 to activate the epithelial barrier and recruit cytotoxic phagocytes. However, unchecked activation of this pathway can lead to inflammatory skin diseases such as psoriasis. Here we describe the TGF- $\beta$  coreceptor CD109 as a key checkpoint in controlling the IL-23/IL-17 immune axis in the skin. We find that mice genetically deficient in CD109 spontaneously activate IL-23 expressing dendritic cells in the skin that leads to accumulation of IL-17 producing  $\gamma\delta$  T cells, epidermal hyperplasia and dermal neutrophilia. The absence of CD109 also exacerbates psoriatic-like dermatitis upon immune challenge and expression of CD109 by radioresistant cells alone is sufficient to recapitulate dysregulation of the skin immune compartment. Collectively, these results reveal CD109 as a stromal-derived rheostat that limits the cutaneous IL-23/IL-17 axis to maintain balance at this critical barrier site.

#### P042 | Identification of parameters that could predict time to return of symptoms after stopping omalizumab treatment: exploratory analysis of Phase III data from patients with chronic idiopathic/spontaneous urticaria (CIU/CSU)

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**Background:** In patients with CIU/CSU, who remain symptomatic despite use of antihistamines, omalizumab, a humanized recombinant monoclonal anti-IgE antibody, significantly improved outcomes in three Phase III randomized clinical trials. We aimed to identify characteristics that could predict the speed of symptom recurrence following treatment discontinuation. We also explored the potential relationship between the timing of response onset and the speed of symptom recurrence in patients with CIU/CSU treated with omalizumab.

**Methods:** This exploratory analysis was performed on pooled patientlevel data from the ASTERIA I (n=319) and ASTERIA II (n=323) trials. Patients received omalizumab 75, 150, 300 mg or placebo q4w for 24 weeks (ASTERIA I) or 12 weeks (ASTERIA II). Follow-up periods lasted for 16 weeks and a LASSO regularization regression model was used to select variables predictive of relapse over this period. Least squares linear regression with prediction intervals was used to estimate the relapse probability for each patient based on the selected variables. Heat map visualizations were used to represent the variation of probability of relapse with the selected variables.

**Results:** The LASSO model identified two parameters that can jointly yield a probability of a patient's recurrence of symptoms after treatment discontinuation: the area above the curve (AAC) of the Urticaria Activity Score summed across 7 days (UAS7) over the initial 4-week treatment period; and baseline UAS7. The results suggest that a low baseline UAS7 and a high AAC (fast initial response) indicate that a

slow return of symptoms is probable upon discontinuation, whereas a high baseline UAS7 and a low AAC (slower initial response) indicate a higher probability of fast return of symptoms.

**Conclusion:** This analysis suggests that it may be possible to estimate the probability of return of symptoms after discontinuing omalizumab treatment in patients with CIU/CSU, based on the baseline UAS7 and the AAC for the early response to omalizumab treatment. A better understanding of the potential clinical relevance of this exploratory analysis is needed.

#### P043 | Omalizumab effectively reduces angioedema episodes in patients with chronic idiopathic/spontaneous urticaria (CIU/CSU)

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**Background:** Phase III trials of omalizumab have already shown high efficacy on wheal and itch in CIU/CSU. However, the long-term effect on histamine-induced angioedema is less clear. This German "X-ACT" Phase IIIb, double-blind, placebo-controlled study investigated the effect of omalizumab on patients with CIU/CSU with frequent angioedema attacks, who are not treated sufficiently with up to 4x standard doses of second-generation H-antihistamine.

**Method:** Patients with CIU/CSU with frequent angioedema received either omalizumab 300 mg (n=44) or placebo (n=47) every 4 weeks for 7 months. The primary efficacy endpoint was the change from baseline in quality of life (assessed by CU-QoL) at Week 28. The secondary endpoint "angioedema" was assessed by the number of angioedemaburdened days.

**Results:** Overall, 91 patients received ≥1 dose of study drug (results are shown as: mean [standard deviation]): age 42.9 [12.3] years; baseline CU-QoL: omalizumab 55.4 [13.6], placebo: 56.1 [17.2]. At baseline, 9% of patients suffered from daily angioedema, 49.5% suffered from weekly episodes and 36.3% suffered from more than six angioedema episodes during the year before baseline. During 28 weeks of omalizumab treatment, the CU-QoL significantly improved to 20.3 [21.8] points vs 42.3 [23.0] in the placebo group (P<.001). Also, during study treatment the number of angioedema-burdened days was considerably less in the omalizumab group (14.6 [19.5 days] compared with the placebo group (49.5 [50.8]). Furthermore, omalizumab-treated patients showed a longer time period between the first and second angioedema episodes following treatment, 20 [41.63] days vs 7.8 [14.29] days in placebo-treated patients.

**Conclusion:** Compared with placebo, add-on of monthly omalizumab 300 mg significantly reduced the occurrence of angioedema in patients with CIU/CSU highly burdened with angioedema.

#### P044 | Omalizumab normalizes gene expression in lesional skin of patients with chronic idiopathic/spontaneous urticaria: results from a randomized, double-blind, placebo-controlled study

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**Background:** Chronic idiopathic/spontaneous urticaria (CIU/CSU), including severe disease refractory to current therapies, shows a strong response to omalizumab, a humanized recombinant monoclonal anti-IgE antibody. Omalizumab's effect on gene expression was assessed in skin biopsies from patients with CIU/CSU enrolled in a double-blind, placebo-controlled study (NCT01599637).

Method: Patients with CIU/CSU (18-75 years) were randomized to either omalizumab 300 mg (n=20) or placebo (n=10) administered subcutaneously every 4 weeks for 12 weeks. Lesional and non-lesional skin biopsies were collected from the same body area of consenting subjects and assessed at baseline and Day 85. Skin biopsies from the same area of ten untreated healthy volunteers were also processed as reference. Gene-expression data were generated using Affymetrix Human Genome U133 Plus 2.0 Arrays. All statistical analyses were performed using R suite statistical software. In brief, after normalization, low-intensity transcripts (probe sets with intensities <100 in ≥50% of samples) were filtered out. To identify transcriptional changes, linear models were constructed taking into account the type of biopsy, the study visit and the treatment for each patient. Thresholds for statistical significance and minimal fold change (FC) were defined as  $P \le .05$  (no multiple testing correction) and absolute FC  $\geq$ 1.5, respectively.

**Results:** At baseline, 63 transcripts were differentially expressed between lesional (n=25) and non-lesional (n=27) skin. Two thirds of this "lesional signature" was also differentially expressed between lesional and healthy volunteer skin. Upon treatment with omalizumab, >75% of this lesional signature changed to reflect non-lesional skin expression levels (different to placebo, *P*<.01). Transcripts upregulated in lesional skin (compared to non-lesional and/or healthy volunteer skin) suggest increased mast cell/leukocyte infiltration (FCER1G, C3AR1, CD93, S100A8 and S100A9), increased oxidative stress (SOD2), vascularization (CYR61) and skin repair events (KRT6, KRT16A).

**Conclusion:** Omalizumab reverted transcriptional signatures associated with the CIU/CSU lesion phenotype to reflect non-lesional/ healthy volunteer expression levels. This result is consistent with omalizumab-mediated clinical improvement observed in patients with CIU/CSU.

#### Experimental Dermatology –WILEY

#### P045 | Antibiotic exposure limited to early life causes intestinal dysbiosis and exacerbation of skin and lung pathology in murine experimental systemic sclerosis

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**Background:** Intestinal dysbiosis has been shown to influence the outcome of autoimmune disorders. Patients with systemic sclerosis (SSc) have an altered intestinal microbiota. However, its role in SSc pathogenesis remains unknown. We hypothesized that dysbiosis induced by antibiotic (ATB) exposure will influence skin and lung pathology development in Topoisomerase I peptide-loaded dendritic cells (TOPOIA DCs)-induced SSc-like disease characterized by progressive and protracted skin and lung fibrosis.

**Methods:** Breeders were exposed to streptomycin and weaned pups continued to receive streptomycin until endpoint (lifelongATB) or discontinued one week before start of immunization (earlyATB). Alternately, adult mice were administered streptomycin one week prior to start of immunization (adultATB) until endpoint. TOPOIA DCs (no streptomycin) immunized mice served as controls.

Results: TOPOIA DCs induced skin and lung fibrosis with upregulated profibrotic IL13 expression. Antibiotic exposure caused differential intestinal dysbiosis and disease outcome in skin and lungs. Specifically, streptomycin administration during any period of life increased skin inflammation, TSLP and MMP12 gene expression, both implicated in SSc and, did not result in lung protection. In adultATB, enrichment of Unc. Lachnospiraceae and Oscillospira sp. was associated with aggravated skin but not lung pathology, upregulated expression of profibrotic (IL13, aSMA, TweakR) and vascular dysfunction (Serpine1, Vwf) genes. However, lifelongATB, that favoured increased Bacteroides caccae and Parabacteroides sp., was associated with skewed IL33/TGFB profibrotic gene expression in skin and exacerbated lung fibrosis. Remarkably, streptomycin exposure limited to early life (earlyATB), before immunization, is sufficient to cause sustained intestinal dysbiosis with over representation of Actinobacteria, increased skin IL13 and aggravated lung fibrosis.

**Conclusion:** TOPOIA DCs induced SSc-like disease was associated with IL13 profibrotic pathway in skin and lungs. Manipulation of intestinal microbiota with streptomycin differentially aggravates pathology in these two distinct mucosal compartments, with early life being a critical window to influence disease outcome. In conclusion, this study links intestinal dysbiosis and SSc pathogenesis and opens doors for evaluating therapies aimed at restoring a normal microbiota in SSc patients.

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#### P046 | CD8+ polycytotoxic T cells are defined by natural killer cell receptors and exhibit antimicrobial activity against intracellular bacteria

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CD8+ cytotoxic T cells (CTL) contribute to antimicrobial defense against intracellular pathogens, yet their heterogeneous expression of the key cytotoxic granule proteins perforin, granulysin and granzyme B, has precluded direct functional study. To determine which subset of CD8+ CTL deliver the crucial payload to kill or inhibit intracellular pathogens, we studied leprosy as a model of infection due to the intracellular pathogen Mycobacterium leprae. We determined that a subset of CTL that co-express all three cytotoxic molecules, polycytotoxic T cells (P-CTL), is increased in the resistant (tuberculoid leprosy. T-lep) vs the disseminated (lepromatous leprosy, L-lep) form of the disease. RNA-seg analysis of P-CTL identified a gene signature that included an array of 14 modulatory NK surface receptors. Further investigation demonstrated that P-CTL are a subset of CD3+ CD8+ T effector memory cells expressing diverse TCRs as well as IL2Rß, facilitating their expansion in response to IL-15. P-CTL, characterized by co-expression of the two NK receptors NKG2a and NKG2c, exhibited a greater antimicrobial activity against intracellular M. leprae as compared with other CTL populations. Together, these data define a functional subset of CD8+ CTL, P-CTL, which contributes to antimicrobial host defense against an intracellular bacterium.

#### P047 | Inhibition by oral administration of Lactobacillus pentosus KF340 of atopic dermatitis-like symptoms induced by house dust mite in mouse: Control of splenic B10 cell differentiation by lactic acid bacteria

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Recently, lactic acid bacteria (LAB) have been proposed as an effective suppressor of allergic symptoms. In particular, biological efficiency of certain LAB isolated from fermented foods made of vegetables has been highlighted because plant-derived LAB are more resistant to gastric juice and bile than animal-derived LAB. Lactobacillus pentosus KF340 (LPKF340) is a bacterium originally derived from the traditional fermented Korean food made mainly of cabbage or radish. It has been reported that L. pentosus is a potent stimulator of IL-10 production but little is known in the context of atopic dermatitis (AD). In present

study, the immunomodulatory effects of LPKF340 were tested in a mouse model of allergic AD. Prior treatment with LPKF340 could effectively alleviate pathologic AD symptoms induced by dust mite extract, including edema and erythema, and decreased lymphocyte infiltration into the inflamed region. In the LPKF340-fed group, the level of allergic cytokine, IL(interleukin)-4, was reduced, while that of anti-inflammatory IL-10 was increased. It was found that splenic regulatory IL-10-producing B cells (B10 cells) were stimulated to produce IL-10 by dendritic cells (DCs) of Peyer's patch. This was dependent on the activity of B cell activating factor (BAFF) in DCs. In conclusion, oral administration of LPKF340 isolated from kimchi can prevent AD through systemic enhancement of IL-10 production from splenic CD1d+CD5+B10 cells. Therefore, LPKF340 may provide a safe and effective means of preventing AD.

#### P048 | Streptococcus pyogenes activation of circulating CLA+ T cells in guttate and chronic plaque psoriasis patients: IL-9 production dependence on time of flare, PASI, ASO levels, and relationship with IL-17 response

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Pharyngeal Streptococcus pyogenes infection can trigger both guttate and chronic plaque psoriasis forms. At present, the immunological mechanisms behind such innate-induced adaptive immune response can be addressed by the ex vivo coculture of circulating memory CLA+ T cells together with autologous epidermal cells activated by Streptococcus pyogenes extract (SE). In this study we have analyzed the response to SE in untreated guttate (n=16) and plaque psoriasis (n=12), and in healthy controls (n=10). Our results indicate that CLA+ T cell-dependent cytokine response in guttate patients ex vivo is related to clinical status of the patients such as the time elapse since flare, PASI, and ASO level, with a peak at 1-2 months. We have detected IL-9 production in both guttate and plaque psoriasis that depends on CLA+ and epidermal cell interaction, and through HLA class I (50%) and class II (90-100%), together with a preferential IL-17A response over IFN-y in gutatte compared to chronic plaque. Neutralization of IL-9 decreased SE-induced IL-17A response (50%) without affecting IFN-γ production. In a 5 days time-course assay IL-9 amounts paralleled IL17A and IFN-y and were the highest at end of culture. Interestingly, CLA-dependent SE response in chronic plaque psoriasis is less dependent on ASO and time after flare, since it is a chronic situation, but presents a mixed Th1/Th17/Th9 response to SE. These results indicate that in psoriasis, the innate activation induced by SE leads to IL-9 production that supports IL17-A production. Such activation depends on CLA+ T cell/epidermal cell interaction and

HLA-mediated presentation. In guttate psoriasis the IL-9 production relates with clinical features of the patients. The induction of IL-9 by a clinically relevant trigger of psoriasis through skin-specific memory T cells, and its relationship with IL-17A production, supports the clinical translational relevance of these results for psoriasis.

## P049 | Targeted therapy in auto-inflammatory diseases: A successful case of aseptic abscess syndrome

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Auto-inflammatory diseases are a group of rare syndromes associated with autoreactive activation of pro-inflammatory cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , or IL-6. While each of these cytokines may be specifically blocked by therapeutic antibodies, personalized medicine is not yet established in the field. We present a 35 year old female that admitted with chronic relapsing, dolorous cutaneous abscess formations on the legs, mandible, liver and skin folds. Further, she complained about fever, abdominal pain, arthralgia and malaise. Repeated swaps of ichor and lesional skin as well as blood cultures remained sterile. The patient underwent multiple adapted antimicrobial and antiseptic therapies without improvement. A skin biopsy showed neutrophilic suppurating inflammation without granuloma or signs of vasculitis. Finally, a corticosteroid pulse therapy (prednisolone) was initiated with prompt remission of cutaneous and constitutional symptoms. However, withdrawal of the drug resulted in further abscesses and flare-ups of polyarthritis and abdominal pain - even with concomitant infliximab treatment. Steady but undulant elevation of inflammatory markers (C-reactive protein, blood sedimentation rate, white blood count) pointed to an auto-inflammatory disease, compatible with aseptic abscess syndrome. Mutation analysis displayed polymorphism of the proline-serine-threonine phosphatase-interacting protein 1 (PSTPIP1) gene, also known as CD2-binding protein 1 (CD2BP1). As a regulatory phosphatase protein, it modulates cytoskeletal organization, T-cell activation and production of IL-1. It has been proposed that polymorphisms may play a role in aseptic abscess syndrome and predispose individuals for protracted inflammatory process. Following this rationale, a change from infliximab to anakinra was made and led to improvement, but incomplete remission. Since in this special case high amounts of IL-6 were detected in sera, the patient received tocilizumab in routine intervals. Within weeks a stable clinical remission set in.

The presented case illustrates, that along with a deeper immunological understanding of the pathogenesis of auto-inflammation, individual profiling of acute phase cytokines can provide the basis for targeted therapy in otherwise difficult to treat auto-inflammatory diseases.

## P050 | TNIP1 loss sensitizes post-receptor signaling following exposure to TLR agonists

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Tumor necrosis factor  $\alpha$ -induced protein 3-interacting protein 1 (TNIP1) restricts cytoplasmic signaling by trans-membrane receptors, such as Toll-like receptors (TLRs), TNF- and EGF-receptors, and transcriptional activity of nuclear receptors PPAR and RAR. However, downstream consequences of this signaling repression are only partly understood. Here we define gene expression and cytokine secretion effects due to TNIP1 regulation of TLR signaling pathways in keratinocytes. In addition to signaling in cutaneous immune cells, TLR activation in non-immune cells such as keratinocytes is likely to contribute to skin inflammatory disease. Our previous microarray analysis with HaCaT keratinocytes showed increased TNIP1 reduces transcription of IL-6 and IkBa, key pro-inflammatory mediators downstream of TLR activation. Additionally, global expression analysis showed immune and inflammatory signaling pathways are significantly affected. Together, these responses from TNIP1 excess predict its loss, as seen for some cutaneous inflammatory pathologies, should prime cells for response to TLR agonists. The purpose of this study was to define specific cytoplasmic targets for TNIP1 and follow these to inflammatory cytokine and chemokine gene expression outcomes downstream of TLR signaling. We modeled TNIP1 loss in HaCaT keratinocytes by siRNA knockdown; its decreased expression was confirmed via gRT-PCR and western blotting. TNIP1 loss increased basal expression of most TLRs examined, as well as IkBa, and their associated downstream pro-inflammatory cytokines, IL-6, IL-8 and TNFa. Compared to control cells, TNIP1-deficient HaCaT keratinocytes predominantly responded to TLR3 (poly I:C) and TLR2/6 (FSL-1) agonists, as measured by IL-6 and IL-8 expression. We found the poly I:C response is due mainly to exaggerated p38 phosphorylation and nuclear translocation of transcription factor NF-KB; the FSL-1 response is due to exaggerated JNK phosphorylation. In conclusion, TNIP1 loss affects not only post-TLR signaling pathways, evidenced by increased cytokine secretion, but also receptor expression itself, revealing multiple levels of TNIP1 regulatory impact. Its importance in restricting inflammatory signaling control suggests that TNIP1 may be an attractive target for drug development.

#### P051 | Induction of remission and cytokine levels in pemphigus after selective plasma exchange therapy

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Systemic steroids, azathioprine, mycophenolate mophetil/ mycophenolate sodium are standard treatment agents in pemphigus. In -WILEY—Experimental Dermatology

refractory cases, intravenous immunoglobulin, rituximab, cyclophosphamide, extracorporeal photopheresis, plasmapheresis and immunoadsorption can be used. Still some patients can be refractory to all these treatment modalities. Here two severe recalcitrant patients are presented who have responded dramatically to selective plasma exchange therapy.

**Case-1:** This patient had pemphigus vulgaris with severe mucosal and cutaneous erosions and was refractory to high dose systemic steroids, adjuvant azathioprine 100 mg daily, cyclophosphamide 2.5 mg/kg daily, intravenous immunoglobulin 2 g/kg every two weeks, extracorporeal photopheresis and double filtration plasmapheresis. (Rituximab could not be given due to serious infections). He had severe septicemia with multi-organ insufficiency therefore selective plasma exchange therapy (SPET) was applied for the treatment of septicemia. After several cycles of SPET, his pemphigus dramatically improved. He died due to complications of septicemia.

**Case-2:** This patient had severe pemphigus foliaceus with >60% skin involvement and was refractory to high dose steroids, adjuvant azathioprine, extracorporeal photopheresis every two weeks and intravenous immunoglobulin at a dosage of 2 g/kg every two weeks. Cyclophosphamide or rituximab could not be given due to serious infection. This patient also had severe septicemia with multi-organ insufficiency and had applied SPET. After several cycles of SPET, he also had dramatically improvement clinically. He had died due to complications of septicemia.

**Conclusion:** Enhanced cytokine levels in serum of pemphigus vulgaris patients have been reported before. We observed, two recalcitrant patients with severe septicemia and extremely high cytokine levels leading to multiple organ insufficiency. SPET (a serum cytokine lowering treatment) induced dramatic clinical improvement in both patients, although the titer of pemphigus antibodies were as high as 1/1280 after SPET. This observation suggested that serum cytokine levels could have an important role in pathogenesis and/or disease activity.

#### P052 | Proseek multiplex: A precision proteomics solution for targeted human protein biomarker discovery

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In the inflammatory skin disease field, as in many others, there is a pressing need for more biomarkers. Identifying relevant protein biomarkers helps to bring new insights into disease processes, improve disease detection, and contribute to a better understanding of pathophysiology. Using multiple proteins that form a signature is more powerful and reliable than looking at a single protein, but this requires studies that examine many different proteins simultaneously, in large numbers of human samples. Proseek<sup>®</sup> Multiplex enables such studies by providing sensitive, high-multiplex immunoassays that do not compromise on data quality. We will show how our dual-recognition, DNA-coupled Proximity Extension Assay (PEA) technology overcomes

the cross-reactivity problems normally associated with multiplexed immunoassays, providing exceptional readout specificity. Details of the thorough validation procedures we apply to all of our assays will be presented, along with the design strategy behind our collection of 92-plex, disease-focused biomarker panels, which now includes a new panel focused on immuno-oncology.

#### P053 | Interleukin-13 in cutaneous T-cell lymphoma: The missing link between inflammation and malignancy

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**Background:** Cutaneous T-cell lymphomas (CTCL) clinically and immunologically remarkably resemble inflammatory dermatitides. Like atopic dermatitis (AD), CTCL is characterized by Th2-driven inflammation, where IL-4 and IL-13 cytokines play a key role. IL-13 is highly expressed by several tumors and acts as a growth factor for tumor cells. Here we analyzed global stage-related immunologic abnormalities in CTCL, the role of IL-13 and its signaling molecules in CTCL pathogenesis, and IL-13's potential to serve as a target for therapeutic use by anti-IL13 antibodies. The overlapping features of benign and malignant inflammatory dermatoses offer an opportunity to investigate into similar therapeutic approaches.

**Methods:** Global assessment of soluble proteins in CTCL was performed using Luminex high throughput technology. Expression of IL-13 and its receptors by CTCL skin tumors and leukemic cells were evaluated by immunohistochemistry, confocal immunofluorescence microscopy and flow cytometry. Analysis of tumor cell proliferation after neutralization of IL-13 and its signaling pathways was determined by MTT assay as well as by CFSE staining.

**Results:** We observed global immune dysregulation in patients with CTCL similar to AD. Increase in IL-5, eotaxin, MIP1B associated with eosinophilia and Th2 skewing showed especially strong associations with advanced stages of the disease in CTCL patients. IL-13 and its receptors IL-13Rα1 and IL-13Rα2 were highly expressed in the lesional skin of CTCL patients, particularly in advanced-stage disease. Malignant lymphoma cells, identified by the co-expression of CD4 and TOX in the skin and blood of CTCL patients, produced IL-13 and expressed both receptors. Furthermore, tumor cell proliferation was inhibited by neutralization of IL-13 through anti-IL-13 monoclonal antibodies or soluble IL-13Rα2 molecules, and by blocking the IL-4/IL-13 signaling pathway.

**Conclusions:** IL-13 and its signaling mediators are novel molecular markers of CTCL malignancy. Moreover, IL-13 is implicated as a possible autocrine factor for CTCL. We conclude that IL-13 and its signaling molecules represent markers for the early diagnosis of CTCL and potential therapeutic targets for intervention.

#### P054 | Isolation of anti-psoriatic activities from actinidia arguta: Suppression of imiquimodinduced psoriasis by modulating neutrophilinfiltration to skin

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Psoriasis is a chronic inflammatory skin disease associated with hyperproliferation of keratinocytes, scaly plaques and erythema. Recent studies highlight the importance of neutrophil infiltration to skin as they are directly recruited by abnormal keratinocytes through C-X-C motif ligand (CXCL) chemokines and antimicrobial peptides (AMPs). Indeed, reducing infiltration of neutrophil to skin can be a viable therapeutic approach to psoriasis. Currently, topical corticosteroids are one of the most widely used treatments for psoriasis but they are associated with various side effects such as skin thinning and skin irritation. Thus, development of safe and effective topical reagents for psoriasis are in need. We have previously shown that PG102, a water-soluble extract from Actinidia arguta, also known as hardy kiwifruit, suppress atopic dermatitis in both animal models and human patients. Here, we show that topical application of PG102 alleviates imiquimod-induced psoriasis in Balb/c mice through reduction of neutrophil infiltration to skin. In PG102-treated group, the epidermal thickness, erythema and interleukin (IL)-17A secretion by lymphocytes in the draining lymph node (dLN) were decreased compared to the vehicle-treated group. PG102 treatment significantly downregulated mRNA expression of CXCL1, CXCL3, CXCL5 and CXCL8 (IL-8) and protein secretion of IL-8 in cytokine-stimulated HaCaT cells. Moreover, mRNA expressions of AMPs such as S100A7 and hCAP-18 which are known to be overexpressed in psoriatic plaques, were also reduced by PG102 in HaCaT cells. Immunohistochemistry of skin tissue with lymphocyte antigen 6 complex locus G6D (Ly6G), the surface marker of neutrophils, showed that neutrophil infiltration was markedly reduced in PG102-treated mice. Taken together, PG102 may provide efficacious means of relieving symptoms of psoriasis without adverse effects, in part by inhibiting neutrophil infiltration to skin.

#### P055 | GILZ overexpression increases psoriasislike features and involves skin TGF- $\beta$ 1 activation in an imiquimod-induced psoriasis mouse model

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Psoriasis vulgaris is a chronic relapsing inflammatory skin disease which affects 2-3% of the world population. Its pathophysiology is complex and is characterized by keratinocyte hyperproliferation, defective differentiation of the epidermal barrier and skin infiltration of immune cells which produce cytokines, eg belonging to the IL-23-Th17-cell axis. One of the most common therapeutical treatment used in diverse cutaneous inflammatory disorders are the glucocorticoids (GCs). GCs anti-inflammatory effects are mediated through the glucocorticoid receptor (GR). GC-induced leucine zipper (GILZ) is a direct target gene of GR that has emerged as an interesting mediator of GCs due to its anti-inflammatory actions, theoretically lacking GC side-effects.

We assessed whether GILZ is able to provide a better therapeutic index in comparison to GCs during the onset and progression of psoriasis by generating and characterizing a mouse model with generalized overexpression of this protein (GILZ-Tg mice) and the imiquimod (IMQ) psoriasis model. Dorsal skin and gut were collected on day seven of IMQ treatment and used for biochemical and histopathological analysis. Cytokines mRNA expression was determined from dorsal skin and gut extracts by RT-qPCR and TGF- $\beta$ 1/SMAD signaling by western blotting.

Unexpectedly, GILZ-Tg mice displayed severe IMQ-induced psoriasislike skin features (erythema and scaling) compared to GILZ-Wt, as well as higher significant induction of cytokines commonly up-regulated in human psoriasis (II17, II22, II23, II6, S100a8/a9, and Stat3) relative to GILZ-Wt mice. This increased susceptibility to IMQ-induced psoriasis of GILZ-Tg mice was significantly associated with skin-specific overactivation of TGF-β1-mediated signaling via SMAD2/3.

Our results suggest that GILZ show pro-inflammatory effects in certain tissues and as an alternative treatment for psoriasis, GILZ may also have side-effects, equal to prolonged GC therapy.

## P056 | Langerhans cells can infiltrate primary cutaneous melanomas and display a tolerogenic phenotype

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Langerhans cells (LCs) are antigen-presenting cells that reside in the epidermis and can direct adaptive immune responses locally by promoting regulatory T cells (Tregs). Melanoma is a malignancy that arises in the epidermis and LCs may therefore play an important role in modulating local anti-tumor immune responses. In order to investigate whether LCs are associated with Treg accumulation in melanoma, we assessed LC co-localization with immunosuppressive Tregs as well as LC inhibitory and stimulatory receptor expression in situ in primary melanoma lesions, healthy skin and benign nevi. Histological sections underwent immunofluorescent staining for the LC markers -WILFY-Experimental Dermatology

Langerin and CD1a, and other surface receptors. In a number of melanoma patients, LCs were found to infiltrate the tumor tissue and co-localize with CD3+ T cells. Similarly, LC infiltrated benign nevi and the surrounding epidermis, but only rarely the dermis of healthy skin. The tumor-infiltrating LCs were less likely to express the activation marker CD40 compared to dermal LCs in normal skin or LCs infiltrating benign nevi, but were more likely to express the inhibitory receptor PDL-1. However, melanoma-infiltrating LCs did not express the co-stimulatory receptor CD80. Interestingly, an increase in FoxP3+ Tregs as percentage of CD4+ T cells could be observed among melanoma samples compared to healthy skin. In conclusion, LCs in primary melanoma lesions appear to have a tolerogenic phenotype.

#### P057 | The leukotriene B4 and its receptor BLT1 act as gatekeeper for neutrophil recruitment in murine pemphigoid disease-like skin inflammation

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Recruitment of neutrophils and eosinophils into the skin is a hallmark of skin inflammation in pemphigoid diseases. The molecular cues regulating granulocyte recruitment into the skin as well as the individual contribution of neutrophils and eosinophils to pemphigoid disease are, however, poorly understood. The lipid mediator leukotriene B4 (LTB4) is a potent granulocyte chemoattractant and abundant in the skin blister fluid of bullous pemphigoid disease patients, but its pathogenic significance is unknown. Using a mouse model of bullous pemphigoid-like epidermolysis bullosa acquisita, we show that LTB4 and its receptor BLT1 act as gatekeepers for neutrophil entry into the skin. Mice deficient in 5-lipoxygenase, a key enzyme in LTB4 biosynthesis, or in BLT1 exhibited dramatic resistance to neutrophil recruitment and, consequently, skin inflammation. Accordingly, mass spectrometry, comprehensively profiling lipid mediator generation in the first 48 hours after antibody deposition, revealed a pronounced parallel increase in LTB4 and in neutrophils in the skin. Subsequent experiments uncovered that solely neutrophils are necessary for the emergence of skin inflammation, while eosinophils are dispensable, thus identifying neutrophils as major culprits of blister formation. Our results highlight the LTB4/ BLT1 axis as absolutely critical driver of pemphigoid disease-like skin inflammation and, hence, as promising therapeutic target.

#### P058 | Immunological analysis of metastatic melanoma patients treated with nivolumab; is Th9 cell subset a possible biomarker of treatment efficacy?

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Anti programmed cell death 1 (PD-1) antibody, nivolumab, leads to a significant improvement in overall survival and progression-free survival, but only 20 to 40% of patients experience long-term benefit. It is therefore of great interest to find a parameter that can be validated as a surrogate marker of response or survival benefit.

To address this issue, frequencies of CD4+ T cell subsets (regulatory T cells, T helper (Th) 1, 2, 9, 17, and 22 cells), CD8+ T cells, and serum cytokine levels (interferon (IFN) - $\gamma$ , interleukin (IL) -4, IL-9, IL-10, and transforming growth factor (TGF) - $\beta$ ) were assessed in 46 melanoma patients with melanoma. Eleven patients responded to nivolumab, and the other 35 patients did not. An early increase in Th9 cell frequency during the treatment with nivolumab was associated with an improved clinical response. Before the first nivolumab infusion, the responders displayed elevated serum concentrations of TGF- $\beta$  compared to the non-responders. Th9 induction by IL-4 and TGF- $\beta$  was enhanced by the presence of anti PD-1 antibody in vitro.

The role of IL-9 in disease progression was further assessed using a murine melanoma model. In vivo IL-9 blockade promoted melanoma progression in mice using an autochthonous mouse melanoma model, and the cytotoxic ability of murine melanoma-specific CD8+ T cells was enhanced in the presence of IL-9 in vitro.

These findings suggest that Th9 cells, which produce IL-9, may play an important role in the successful treatment of melanoma patients with nivolumab. Th9 cells therefore may represent a valid biomarker to be further developed in the setting of anti PD-1 therapy.

## P059 | Autoimmune comorbidities among 10 psoriatic patients

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**Introduction:** Psoriasis is a common, chronic, immune-mediated inflammatory skin disease affecting 2-3% of population. It is associated with several diseases and approximately 30% of psoriatic patients have psoriatic arthritis. Other common comorbidities include hyperlipidaemia, hypertension, obesity, Crohn's disease and depression/ anxiety. Some autoimmune diseases like rheumatoid arthritis are linked to psoriasis.

**Objectives:** Our aim was to determine autoimmune diseases among 10 patients with severe psoriasis.

**Patients and material:** Ten patients with severe psoriasis (4 women, 6 men) aged 26-74 years (mean age 57,8 years) were referred to the department of oral and maxillofacial diseases for oral examination and eradication of infection foci between July 2015 - March 2016 from the department of Dermatology. Anamnestic information of health, medications and smoking were recorded.

**Results:** Autoimmune diseases were found in four out of 10 patients having altogether seven different autoimmune diseases. Two patients

had one autoimmune disease, one had two and one three autoimmune diseases. The most common autoimmune disease was a rare autoimmune blistering disease, pemphigoid, affecting two women. Other comorbidities, included psoriatic arthritis (5/10) and hypertension (5/10). Autoimmune diseases and other comorbidities are listed on the table 1. The youngest patients without smoking history (2/10) did not have any comorbidities.

**Conclusions:** Patients with psoriasis indeed had different autoimmune diseases as comorbidities including rare pemphigoid suggesting a possible immune mediated link. Due to the limited number of patients more data is needed to explore connection between pemphigoid and psoriasis diseases or medication as a possible triggering factor. When planning psoriasis medication, autoimmune diseases and other comorbidities should be considered.

#### P060 | Novel promotion of skin innate lymphoid cell activity by CD200R1

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Barrier sites rely on specialised immune cells that distinguish, and appropriately respond to, abundant antigenic material at these sites. Consequently, immune suppression is essential to prevent aberrant immune responses towards innocuous antigens. Innate lymphoid cells (ILCs) are enriched at barrier sites, including the skin, and are hypothesised to be involved in inflammatory diseases such as psoriasis. Although much is known about their development and effector functions, relatively little is known about how their activity may be regulated. CD200 usually imparts a negative regulatory signal through its receptor, CD200R1, and can limit inflammatory pathology. We describe for the first time, CD200R1 expression by ILCs in human and mouse skin, suggesting that these too may be regulated upon interaction with CD200. As such we examined the impact of CD200/ CD200R1 signalling on ILC function.

We characterised CD200R1 expression in mouse ILC subsets and though absence of CD200R1 did not affect the repertoire of skin ILC subtypes, its absence had a profound effect on ILC3 functionality. Remarkably, rather than enhancing ILC3 activity, lack of CD200R1 resulted in reduced production of IL-17 following IL-23 stimulation. This suggested that CD200R1 was required for IL-17 production by ILC3s. We confirmed this by showing reduced IL-23-induced ear skin inflammation in CD200R1-deficient mice. Furthermore, stimulation of CD200R1 using a CD200-Fc fusion protein, augmented ILC3 activity in vitro and, in combination with IL-23, increased ear thickness in an in vivo model of skin inflammation.

These data suggest that CD200R1-signals, positively regulate ILC3 activity, ensuring correct production of the effector cytokine IL-17. Strikingly, these effects are unique to the ILC3 subset, as no positive effect of CD200R1-signalling was seen on any other ILC subset. Our novel data reveal for the first time that CD200R1 can enhance skin inflammation, despite previous studies clearly demonstrating an

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anti-inflammatory role for this pathway. Our data may indicate that inhibition of CD200R1 would prevent IL-17 production associated with a plethora of inflammatory diseases at barrier sites.

# P061 | IL-23 pathway inhibition by risankizumab differentially modulates the molecular and histopathological profile in psoriatic skin compared with ustekinumab

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**Introduction & objectives:** IL-23 plays a major role in psoriasis pathophysiology. Specific inhibition of IL-23 with risankizumab demonstrated superiority to ustekinumab in patients with moderate-to-severe plaque psoriasis (Papp K et al., AAD 2016). We investigated the underlying mechanism by comparing the molecular and histo-pathological profile in skin lesions from patients treated with risankizumab vs ustekinumab.

**Materials & methods:** Lesional skin biopsies obtained in two clinical trials of patients with moderate-to-severe psoriasis, a Phase I study (NCT01577550; N=39; baseline, Week 8 biopsies) of single risankizumab doses (0.01-5 mg/kg) vs placebo and a Phase II study (NCT02054481; N=60; baseline, Week 4 biopsies) comparing treatment with risankizumab (18 mg single injection; 90 or 180 mg at Weeks 0, 4 and 16) vs ustekinumab (45 or 90 mg at Weeks 0, 4 and 16), were assessed for histopathology and transcriptome-wide RNA profiling. Univariate associations were assessed using linear regression.

**Results:** As early as Week 4, risankizumab treatment decreased expression of histopathology biomarkers including K16, Ki67, CD3, lipocalin, CD11c, DC-LAMP,  $\beta$ -defensin 2 and S100A7. In the Phase II study, 50% and 64% of patients on 90 or 180 mg risankizumab, respectively, were graded as excellent improvement vs 27% for ustekinumab. Risankizumab induced a significant decrease (*P*<.005) from baseline to Week 4 in the expression of 1714 unique genes, including IL-23R, CCL20, IL-36 $\beta$ ,  $\beta$ -defensin 4B, and type I IFN pathway and late cornified envelope family genes, in patients who received risankizumab but not ustekinumab. At 4 and 8 weeks, risankizumab strongly downregulated expression of genes associated with keratinocytes and epidermal cells and, to a lesser extent, those associated with monocytes, macrophages, dendritic cells and neutrophils.

**Conclusions:** The superior clinical efficacy of risankizumab vs ustekinumab was associated with early changes in the molecular and histopathological profile of skin lesions in patients with psoriasis. This suggests differences in the mechanistic action of specific IL-23 p19 inhibition compared with inhibition of the combined IL-12 p40/IL-23 p19 subunits. 26 WILEY Experimental Dermatology

#### P062 | Arginase1/iNOS imbalance mediates cutaneous contact hypersensitivity responses

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Macrophages play relevant roles in allergic contact dermatitis (ACD). Using the contact hypersensitivity (CHS) mouse model, a model of ACD, we find that induced depletion of macrophages at the elicitation phase reduces ear thickness (P≤.05), suggesting a critical role of macrophages in this phase of CHS. Arginase1 (Arg1) and inducible nitric oxide synthase (iNOS or Nos2) are important enzymes in macrophages that reciprocally regulate inflammation. Arg1 is expressed at highest levels in dermal macrophages and is associated with anti-inflammatory functions, whereas iNOS confers pro-inflammatory roles. Dysregulation of Arg1 and iNOS in macrophages have been described in several diseases, however their regulation and function in ACD is unknown. Therefore, we investigated roles of Arg1/iNOS in regulating CHS. In vitro stimulation of bone marrow-derived macrophages (BMDM) with DNBS, a hapten allergen, decreased Arg1, but induced Nos2 expression and this response could be normalized by dexamethasone treatment (P≤.05). In the in vivo CHS mouse model, Arg1-/flox;LyzMcre mice develop increased skin inflammation accompanied by increased ear thickness compared to control mice (P≤.05) upon DNFB challenge. Deletion of Arg1 in macrophages also exaggerated the irritant contact dermatitis response (P≤.05), suggesting that Arg1 in macrophages attenuates skin inflammation in both in antigen-dependent and -independent contexts, respectively. Notably, increased Nos2 expression was observed in DNBS-treated BMDM from Arg1-/flox;LyzMcre mice compared to wild-type cells (P≤.05). The functional relevance of increased Nos2 in Arg1-deficient macrophages is supported by the finding that DNFBelicited Arg1-/flox;LyzMcre mice treated with a selective iNOS inhibitor resulted in a significant decrease in ear thickening compared to vehicletreated CHS mice and to wild type controls (P≤.05). Furthermore, iNOS-/- mice had reduced ear swelling compared to wild-type mice in CHS (P≤.05). Together, our data demonstrate that Arg1 in macrophages suppresses CHS through dampening Nos2 and that the immunosuppressive function of dexamethasone may be entailed through modulation of Arg1/Nos2 expression in macrophages. Our findings suggest the Arg1/ iNOS pathway as a potential therapeutic avenue in ACD.

#### P063 | Secukinumab, a new anti-IL17A biologic therapy, induces rapid and durable clinical, histological, and molecular resolution of psoriasis plagues over 1 year of administration

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Secukinumab, a fully human monoclonal antibody that selectively neutralizes IL-17A, has significant efficacy in the treatment of moderate-to-severe psoriasis and psoriatic arthritis, demonstrating a rapid onset of action and sustained responses with a favorable safety profile. CAIN457A2223 (NCT01537432) was a randomized, double-blind phase 2 trial that investigated the effects of a clinically relevant dose of secukinumab over 1 year in moderate-to-severe plague psoriasis. In part I, 36 patients were enrolled to receive either 300 mg subcutaneous secukinumab at Baseline, Weeks (Wks) 1, 2 and 3, and then every 4 weeks, or placebo (2:1), for 12 weeks. In part II, all patients received secukinumab 300 mg every 4 weeks to Wk 52. In addition to clinical assessments (eg, PASI score), lesional plaque and nonlesional skin biopsies were taken at Baseline, Wks 1 or 4, 12, and 52, assessed for histologic improvement and immune cell infiltrates, and mRNA profiled. In part I, 13/23(57%) of secukinumab-treated and 0/12 placebo patients achieved histologic disease reversal by Wk 12 (P<.001). Secukinumab did not affect ex vivo T-cell activation at Wk 4, and resident skin immune cell populations were retained. Consistent with phase 3 trials, 14/24(58%) of secukinumab-treated patients achieved PASI90 by Wk 12, and histological disease reversal correlated with %PASI improvement (r=-.77. P<.001). In part II, 19/36(53%) of patients administered secukinumab for 40 or 52 weeks achieved histologic disease reversal by Wk 52, with 12/13(92%) patients who received secukinumab up to Wk 12 maintaining histologic disease reversal at Wk 52. Transcript profiles of secukinumab-treated lesional skin shifted toward those of nonlesional skin in the majority of patients starting at Wk 1 and continuing through Wk 52, including reductions in IL-17-pathway genes and antimicrobial proteins such as beta-defensin-2. In summary, secukinumab has rapid, progressive, and durable effects on psoriasis skin histopathology and psoriatic gene signatures, confirming that IL-17A is a major driver of pathophysiologic changes in psoriatic skin in a majority of patients.

#### P064 | Periodontitis in oral pemphigus and pemphigoid: A systematic review of published studies

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Periodontitis and autoimmune bullous diseases including pemphigus vulgaris (PV) and mucous membrane pemphigoid (MMP) are immunoinflammatory disorders leading to plague- and autoantibody-elicited tissue injury of the oral cavity, respectively. Evidence implicates

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that these autoimmune conditions may represent a risk factor for periodontitis, but no systematic evaluation is available to corroborate this assumption. We therefore conducted a systematic literature review of the periodontal status in pemphigus and pemphigoid diseases. Electronic searches using the PubMed database from inception to July 2016 identified 10 studies which met our predetermined inclusion and exclusion criteria. Most of these case-control or pilot studies reported that there is some correlation between poor periodontal health and both oral PV and MMP, with some demonstrating beneficial effects of oral hygiene procedures on periodontal parameters as well as severity of the blistering diseases. Some inconsistent results, however, were found between studies and within the analyzed patient cohorts, likely due to methodological shortcomings. Our review preliminarily suggests that patients with oral PV and MMP appear somewhat susceptible to periodontitis and should be encouraged by dermatologists to pursue professional periodontal followup, although the true relation and mutual interaction between both diseases still needs to be more comprehensively addressed in welldesigned prospective studies.

#### P065 | Gender differences in central processing and perception of itch: "Same same but different"—Preliminary results of a study using functional MRI

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**Background:** Itch is the most frequent symptom in patients with inflammatory skin diseases. Its impact on quality of life is comparable to that of pain. Study data based on patient self-reports indicated that women perceive itch more intensively and bothersome as compared to men-raising the question of a gender-tailored itch treatment. However, studies using objective methods to measure itch in that context are lacking. This is the first study to address gender differences using serial subjective itch ratings and functional magnet resonance imaging (fMRI) of the brain following itch induction.

**Methods:** In this double-blinded, within-subject, placebo-controlled study with 15 female and 15 male healthy volunteers itch was induced by a skin-prick with histamine 1% or saline on the right forearm. Itch was rated on a Numerical Rating Scale at 1-minute intervals during 10 minutes whilst being scanned (3Tesla MRI-scanner).

**Results:** In line with previous reports brain activity was observed in the anterior cingulate cortex (ACC), prefrontal cortex (PFC), premotor area, somatosensory areas, insula and cerebellum. Regarding itch rating, size of the induced wheal/erythema no gender difference was found. However, a significant correlation between male gender and brain activity in the ACC and PFC and female gender and activity in the right frontal temporal lobe (Brodmann area BA38) was observed. **Discussion:** Histamine-induced itch did not result in gender differences regarding itch intensity and local reaction. However, men had more activity in areas related to the evaluation of a scratch response (PFC) and emotional-affective aspects of itch (ACC). The BA38 was previously linked to binding perceptual inputs with emotions, but the precise role of this area is unclear. Our fMRI-results will be further analysed by correlation with questionnaires that cover emotional and qualitative aspects of itch perception. Our findings need further elucidation in patients with itch as our itch-model may not represent reallife itch conditions.

**Conclusion:** Gender differences in central itch processing can be objectified by fMRI.

#### P066 | Characterization of multiple B cell subsets in peripheral blood of psoriasis patients identifies a correlation of plasma and regulatory B cells with disease severity

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Imbalances of T cell subsets have been demonstrated as hallmarks of disease-specific inflammation in psoriasis. However, the role of B cells as important counterpart of T cell function remains poorly investigated. Here, we analysed a broad set of B cell subsets and immunoglobulins in psoriasis patients and correlated their distribution in peripheral blood with disease severity.

Surface staining and flow cytometry was performed on leukocytes from whole blood of 100 psoriasis patients and 20 healthy individuals without history of skin disease. The severity of psoriasis was determined by Psoriasis Area and Severity Index (PASI) and patients were classified as PASI low (<5) or PASI high (>10). Developmentally different B cell subsets were defined on the basis of CD24, CD38, CD27 and CD138 expression. The humoral immunologic profile was complemented by serum parameters including immunoglobulins. We found a significant increase of plasma cells accompanied by increased IgA serum levels in patients with higher severity scores (PASI high) as compared to patients of the PASI low group and healthy volunteers. Moreover, frequencies of regulatory B cells were up-regulated in peripheral blood of psoriasis patients and showed positive correlation with PASI. Furthermore, we investigated the frequencies of regulatory B cells in skin by immunofluorescent stainings on paraffin-embedded skin biopsies from psoriasis patients. Previous data indicate also in the skin a trend for an increase of the numbers of regulatory B cells with increasing disease severity.

These data suggest a contribution of B cell subsets to the severity of psoriasis with increased frequencies of regulatory B cells representing

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a possible compensatory mechanism to increased frequencies of plasma cells and IgA serum levels observed in psoriasis patients.

#### P067 | A longitudinal study of the association between preschool eczema or filaggrin mutations and IgE sensitization up to 16 years of age

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**Background:** Children with eczema and/or who carry a filaggrin (FLG) mutation have an increased risk for having IgE antibodies suggesting an allergic sensitization through an impaired skin barrier.

**Objective:** We explored the longitudinal relationship between preschool eczema and/or FLG mutation and IgE sensitization up to 16 years of age. Secondly, we sought to investigate if the allergenspecific IgE pattern differed among sensitized children with or without preschool eczema or FLG mutation.

**Methods:** A total of 3201 children from the Stockholm BAMSE birth cohort were enrolled. At 1, 2, 4, 8, 12 and 16 years of age their parents answered questionnaires regarding eczema in their child the last year, and at 4, 8 and 16 the children were invited for clinical examination including blood sample for determination of specific IgE against common inhalant and food allergens. Analysis of FLG mutation was performed on 1890 of the children.

**Results:** Preschool eczema was associated with any IgE sensitization at 4 (adjOR 2.51; 95% CI: 2.09-3.03), 8 (adjOR 2.18; 95% CI: 1.84-2.59) and 16 years of age (adjOR 2.20; 95% CI: 1.86-2.61). Presence or absence of FLG mutation did not have any major impact on the adjusted ORs. FLG mutation was not associated with IgE sensitization at 4, 8 and 16 years. Sensitized children with preschool eczema were characterized by polysensitization (IgE positive to >3 allergens) and had higher prevalence of IgE antibodies to both food and aeroallergens, compared with children without preschool eczema. However, IgE sensitization to milk and house dust mite were equally common in sensitized children with and without preschool eczema. Sensitized children with FLG mutation had a higher prevalence of IgE antibodies to peanut at 4 and 8 years of age, but not to other allergens.

**Conclusion:** Preschool eczema, but not FLG mutation, was associated with any IgE sensitization up to 16 years of age. Sensitized children with preschool eczema were more often polysensitized, but otherwise no specific patterns of sensitization were found.

#### P068 | High circulating miR-146a plasma levels found in obese patients with moderate to severe plaque psoriasis can be reduced by anti-TNFs agents

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**Background:** Some human microRNAs (miR) play an important function as regulator of immune responses. miR-146a, a known important negative regulator of immune responses, is one of the most highly up-regulated miRNAs in psoriatic skin. In addition, miR-146a promotes obesity in part by inducing adipocyte differentiation, and silencing of miR-146a ameliorates obesity in murine models. But the role of miR-146a in the cross-talk between psoriasis or cardiovascular disease is largely unknown.

**Methods:** Single-centre prospective study from a cohort of 300 Caucasian patients without cardiovascular disease and moderateto-severe psoriasis treated with etanercept or adalimumab as first biologic was analyzed (ClinicalTrials.gov Identifier: NCT01753245). Clinical features of cardiometabolic risk factors and psoriasis history were analyzed. miR-146a levels were quantified by RT-PCR using miR103 levels as reference control. Cytokine and chemokine plasma levels were quantified by a 27-multiplex ELISA platform. The population was divided into three groups: normal weight, overweight, and obese patients. Changes in circulating markers after anti-TNF treatment were compared between these groups.

**Results:** In this study, circulating miR-146a plasma levels significantly correlated with some cardiometabolic factors [positive with leptine, hsPCR, SBP and BMI values and negative with adiponectin and HDLc levels] and with circulating cytokines/chemokines as mediators of chronic systemic inflammation (positive with IL-1RA, IL-2, TNF $\alpha$ , IL-17, CCL4, FGF- $\beta$  and negative with IL-4 and CCL11 levels). We observed that miR-146a levels were higher in obese and overweighted patients with psoriasis as compared with patients with normal weight. These features were reversed after treatment with anti-TNF agents. **Conclusions:** High miR146 plasma levels correlated with clinical features and metabolic markers of obesity but this can be reversed by anti-TNF treatment. These findings may help us to decipher new functional relationships between these inflammatory diseases.

#### P069 | Crisaborole demonstrates improvement in quality of life in patients with mild to moderate atopic dermatitis

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Atopic dermatitis (AD), a chronic inflammatory skin disease affecting children and adults, presents with eczematous lesions and intense pruritus. The visible and chronic nature of AD often results in psychosocial difficulties and psychologic comorbidities and has a substantial impact on the finances and quality of life (QoL) of patients, their families, and caregivers. Additionally, AD-associated pruritus significantly reduces the QoL of patients because of sleep disturbance and emotional stress. Crisaborole Topical Ointment, 2%, is an investigational, nonsteroidal, anti-inflammatory, phosphodiesterase 4 inhibitor being evaluated for the treatment of mild to moderate AD. Herein, we report QoL results, a predefined additional endpoint, from 2 identically designed multicenter, double-blind, vehicle-controlled Phase 3 studies evaluating crisaborole ointment in patients 2 years or older. Patients with mild to moderate AD were randomly assigned 2:1 to receive crisaborole:vehicle twice daily for 28 days, with QoL evaluations at baseline (BL) and end of treatment. Day 29. OoL of patients was assessed using the validated Children's Dermatology Life Quality Index (CDLQI) (2-15 years) and Dermatology Life Quality Index (DLQI) (16 years or older). The validated Dermatitis Family Impact Questionnaire (DFI) measured the impact of AD on the family/parents/caregivers of patients 2-17 years old. Each questionnaire consists of 10 questions, with a maximum possible score of 30, with higher scores representing worse QoL. Crisaborole-treated children showed significantly greater improvement in CDLQI at Day 29 (from BL, pooled, crisaborole vs vehicle: -4.6 vs -3.0, P<.001). Crisaboroletreated patients 16 years or older showed significant improvement in DLQI at Day 29 (from BL, crisaborole vs vehicle; pooled: -5.2 vs -3.5, P=.016). Parents/caregivers of crisaborole-treated children reported significant improvement in DFI compared with those of vehicletreated children (from BL, crisaborole vs vehicle; pooled: -3.7 vs -2.7; P=.003). These findings suggest that crisaborole might be a promising, novel topical AD treatment that can improve QoL and reduce burden of disease for patients with mild to moderate AD and their families.

### P070 | E-cadherin and p120ctn expression is lost in hidradenitis suppurativa lesions

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Hidradenitis suppurativa (HS) is a chronic and debilitating inflammatory disorder of the folliculo-pilosebaceous unit (FPSU) with no known cure or consistently effective treatment. The etiology of HS is not understood although genetics, mechanical trauma, dysregulated immune response and defective follicular support are hypothesized to play major roles. Characterization of HS lesions has shown differential cytokine

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and cytokeratin expression, yet much remains unknown. E-cadherin, a calcium dependent adherens junction protein, expression has never been examined in HS lesions. Surprisingly, E-cadherin expression is remarkably absent in the epidermis in 88% of lesions (n=17; P<.05) compared to normal skin. Furthermore, p120ctn, a catenin family protein required for E-cadherin regulation and cell membrane stability, is also significantly decreased or absent in these 85% of these lesions (n=26) compared to normal skin. Dysregulation of p120ctn triggers epidermal hyperplasia and increased inflammation. Further examination of the E-cadherin/p120ctn pathway in HS could lead to advances in our understanding of this devastating disease process.

#### P071 | Long-term safety of crisaborole, a novel, nonsteroidal, anti-inflammatory phosphodiesterase 4 inhibitor, in children and adults with mild to moderate atopic dermatitis

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Atopic dermatitis (AD), a chronic inflammatory skin disease, often requires long-term topical treatment. Available topical therapies have advanced little in the past 15 years and are associated with potential safety concerns. Crisaborole is a novel nonsteroidal, topical, anti-inflammatory phosphodiesterase 4 (PDE4) inhibitor being investigated for the treatment of mild to moderate AD to address the need for a safe and targeted long-term treatment. Herein, we evaluate the long-term safety of crisaborole in patients ≥2 years of age with mild to moderate AD who were included in an open-label extension study. After completing a 28-day Phase 3 pivotal study (AD-301, AD-302) patients who opted to continue treatment (N=517) were enrolled in a multicenter, open-label, 48-week safety study (AD-303). Patients were assessed for AD severity every 4 weeks and treated as needed (Investigator's Static Global Assessment ≥2 [Mild]) with 4-week cycles of crisaborole. Safety measures included assessment of adverse events (AEs), serious adverse events (SAEs), clinical laboratory results, physical examinations, and vital signs. During the pivotal studies and the open-label extension study, 65% of patients reported at least 1 treatment-emergent adverse event (TEAE); most were mild (51.2%) or moderate (44.6%) and considered unrelated to treatment (93.1%). The severity and frequency of TEAEs were well balanced over time, as analyzed across four 12-week treatment periods, demonstrating a favorable safety profile for long-term treatment of crisaborole. Overall, treatment-related AEs were reported by 10.2% of patients; the most frequently reported events were atopic dermatitis (3.1%), application site pain (2.3%), and application site infection (1.2%). None of the 7 WIL FY—Experimental Dermatology

reported treatment-emergent SAEs in the extension study were considered treatment related. During the extension study only nine patients (1.7%) discontinued the study because of TEAEs. No cutaneous adverse reactions, such as application site atrophy or telangiectasia, were reported. A favorable safety profile was demonstrated for the long-term treatment with crisaborole for patients with AD.

## P072 | Dysregulated expression of TSP-1 and its receptor CD47 in psoriasis

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**Background:** Accumulating evidence about the role of Thrombospondin-1 (TSP-1) in immune response has emerged in the last years. Some of these properties are through its interaction with CD47, as the induction of Treg cells. In spite of the importance of TSP-1 not only as anti-angiogenic factor but also as an immunomodulatory molecule, its study in psoriasis is almost negligible.

**Methods:** TSP-1 and CD47 was analysed in skin samples from psoriasis patients and control subjects using RT-PCR and immunofluorescence assays. Expression of these molecules was also evaluated in CD4+ T cells and monocyte-derived DCs (moDcs) by RT-PCR, in the case of moDCs, expression of TSP-1 and CD47 was also evaluated in response to LPS. Peripheral CD4+ T cells from healthy subjects and psoriasis patients were differentiated to Th17 and Treg cells in the presence of human recombinant TSP-1 and anti-CD47 to determine the functional of role of these proteins.

**Results:** Lesional skin of psoriasis express low levels of mRNA TSP-1 and CD47 compared to non-lesional skin and skin from healthy subjects. Immunofluorescence staining showed that infiltrating CD11c+ MHC-II+ cells and CD163+MHC-II+ cells from lesional skin express lower levels of CD47 compared to those cells from non-lesional skin. Peripheral CD4+ T cells express also low levels of CD47. We did not detect differences in TSP-1 expression in peripheral CD4+ T cells and moDCs among patients and controls. However, TSP-1 expression in these cell is lower in those patients with higher index of disease activity evaluated as Psoriasis Area and Index Activity (PASI). TSP-1 inhibited Th17 differentiation and stimulated the differentiation of CD4+ T cells into Treg cells. **Conclusions:** Defects in TSP-1 and its receptor CD47 in immune cells from psoriasis patients may favour the exacerbated inflammatory response in Psoriasis.

#### P073 | ATx201: A novel antibiotic for treatment of skin infections caused by multidrug resistant bacteria

<u>M. Sommer</u> Antibiotx, Hørsholm, Denmark **Objectives:** Topically administered antibiotics are ideal for management of dermatological infections, due to a limited systemic exposure resulting in less selection for resistance in the human microbiota and lower side effects. However, currently available antibiotics for cutaneous application are plagued by high levels of resistance in several clinically epidemic strains as well as rapid selection for de novo resistance during treatment. To address the shortcomings of existing medicines for the management of dermatological infections, we here present a new compound ATx201, which is a potent anti-Gram-positive antibiotic that effectively inhibits the growth of Streptococcus and Staphylococcus.

**Methods:** We characterized the microbiological properties of ATx201 against a panel of bacterial isolates with various resistance profiles using broth microdilution, time-kill kinetics, and mutation rate measurements. Additionally, we characterized its behavior in a murine superficial skin infection using *S. aureus* as the target pathogen. Optimized dermal formulations have been characterized in the minipig for tolerance and drug distribution.

**Results:** ATx201 shows strong antibacterial activity against the tested Gram-positive strains. ATx201 exhibits equal potency against strains resistant to commonly used antibiotics such as mupirocin, fusidic acid and retapamulin. Notably, the occurrence of spontaneous resistant mutants is very rare and forms at a frequency below our detection limit (<10-9). ATx201 is able to reduce colonization of fucidic acid resistant *S. aureus* and MRSA in vivo based on data from the murine superficial skin infection model. Finally, ATx201 is well tolerated and achieves high exposure in the dermis and epidermis.

**Conclusion:** ATx201 has a very attractive antibiotic profile and could provide an effective solution for the management of skin infections caused by multidrug resistant Gram-positive bacteria.

#### P074 | A distinct molecular signature of interface dermatitis as determined by gene expression analysis combined with disease independent histological phenotyping

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Inflammatory skin diseases (ISD) are highly heterogeneous in terms of clinical appearance, histological architecture, pathogenesis and underlying triggers. However, certain histological criteria are consistently shared by several ISDs and are therefore an ideal model for investigation of general mechanisms of skin inflammation. Interface dermatitis (ID), characterized by a dense lymphocytic infiltrate at the dermoepidermal junction in combination with apoptotic basal keratinocytes, is a perfect example for this issue, as it appears in inflammatory skin diseases, like lichen planus (LP), as well as in autoimmune diseases, like cutaneous lupus ervthematosus (CLE). In this study 18 cases of either CLE (n=6) or LP (n=12) were analysed using a novel histology score based on 24 objective criteria, thereby allowing a disease independent ranking of ID severity. Furthermore, we performed whole genome expression analysis of lesional and autologous non-lesional skin biopsies identifying genes correlating with the individual ID severity. A high regulation of chemokine (CXCL2, CXCL13) and T cell receptor genes (CD247) as well as a contribution of the JAK/STAT pathway, revealed by induced network modules analysis, characterizes the molecular signature of ID. This study is a new approach to investigate inflammatory skin diseases using objective histological phenotyping instead of conventional disease terminology, thus demonstrating an advanced way to interpret gene expression data and to understand key pathways of skin inflammation.

### P075 | The role of *Candida albicans* in the pathogenesis of psoriasis

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The skin represents a primary interface between the host and the environment. This tissue is not only a primary site of exposure to pathogens but is also home to a complex microbiota that modulate skin immune homeostasis. Notably, our previous work revealed that defined microbes differentially regulate the skin immune landscape. In clinical settings, shifts in skin microbiota composition have been shown in the context of skin inflammatory disorders, such as atopic dermatitis, acne vulgaris and psoriasis. However, the precise role of cutaneous microbiota in the initiation, control or promotion of skin inflammatory states remains unclear. To clarify the mechanisms by which the skin microbiota influence host immunity under inflammatory conditions, we focused on psoriasis, a common inflammatory skin disorder mediated by both innate and adaptive immune systems.

Murine studies have shown that topical application of imiquimod, a TLR7 agonist, can induce psoriasis-like skin inflammation, mediated by the IL-23/IL-17 axis. Using this experimental model, we found that previous skin association of mice with *C. albicans* but not other skin microbes (eg *Staphylococcus aureus*), significantly enhanced skin inflammation. Notably, *C. albicans* topical association promoted IL-17A production from CD4+ effector T cells in the skin and skin draining lymph nodes. Our work currently explores how adaptive immunity directed against *C. albicans* could contribute to the etiology of this important skin disorder. This work aims to define the causative association between defined microbes and the development of skin inflammatory disorders and may potentially allow for the development of tailored clinical interventions aiming at controlling skin microbiota and/ or aberrant responses to these microbes.

## P076 | Altered lycopene isomer ratio and reduced carotenoid and retinoid concentrations in plasma of atopic dermatitis patients

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In the human organism various carotenoids are present of which, some are retinoid precursors. The bioactive derivatives of these retinoids are the retinoic acids, which can potently activate nuclear hormone receptors like the retinoic acid receptor and the retinoid X receptor. In our study by using HPLC analytical approaches we aimed to assess how plasma carotenoid and retinoid concentrations as well as ratios of their isomers are altered in atopic dermatitis (AD) patients (n=20) compared to healthy volunteers (HV, n=20). We found that plasma levels of the carotenoids lutein (HV 198±68 ng/mL, AD 158±57 ng/mL), zeaxanthin (HV 350±142 ng/mL, AD 236±85) as well as the retinoids retinol (HV 216±89 ng/mL, AD 167±76 ng/mL) and all-trans-retinoic acid (HV 1.1±0.6 ng/mL, AD 0.7±0.5 ng/mL) were significantly lower in AD-patients, while lycopene,  $\alpha$ -carotene and β-carotene levels were comparable. In addition ratios of 13-cis vs all-trans lycopene as well as 13-cis vs all-trans retinoic acid were increased in the plasma of AD-patients indicating an AD-specific 13Cisomerisation. A positive correlation with SCORRAD was calculated with 13-cis vs all-trans lycopene ratio, while a negative correlation was observed with zeaxanthin plasma levels. Based on our results we conclude that in the plasma of AD-patients various carotenoids and retinoids are at lower levels, while the ratio of lycopene isomers is also altered. The higher rate of lycopene and retinoic acid isomerisation products might be a consequence of AD or might result in an altered activation of nuclear hormone receptor signaling pathways and thus maybe partly be responsible for the AD-phenotype and additionally may represent a good plasma marker for AD.

#### P077 | Towards the clonotype analysis of alopecia areata-specific, intralesional human CD8+ T-lymphocytes

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Alopecia areata (AA) is an organ-restricted, CD8+ T cell-dependent autoimmune disease which mainly affects the hair follicle (HF) after collapse of its MHC class I-dependent immune privilege, leading to hair loss and major psychological distress. However, the (likely MHC class I-presented) (auto-)antigen(s) and the clonotype of the pathogenic CD8+ T-cells remain unknown. Therefore, we have adopted the systematic screening of hypervariable T cell receptor (TCR) to identify disease-specific, intralesional CD8+ T-cells in situ by laser -WILEY—Experimental Dermatology

microdissection directly in lesional AA skin, the only method which allows one to distinguish between skin- and HF- infiltrating CD8+ T-cells while identifying their paired  $\alpha$  and  $\beta$  chains. In this long-term endeavour, up to now, we have been able to characterize few TCR  $\beta$ -chains, namely expressing V $\beta$ 7, 12, and 27, and the corresponding  $\alpha$  chains, namely expressing V $\alpha$ 4, 10, and 13, (including CDR regions). of autoreactive, intralesional CD8+ T-cells from the skin of AA patients. However, since the latter vary greatly from patient to patient, we are currently continuing such analysis in order to obtain a more representative idea of which CD8 TCRs are expanded in and around AA HFs in a given patient HLA haplotype. That VB7+ and VB12+ CD8+ T-cells, for which β-chain commercially available antibodies exist, represent the most expanded clones in some AA patients was confirmed by immunostaining for CD8 and the corresponding  $\beta$ -chain in lesional skin sections from AA patients. Once we identify the paired  $\alpha$  and  $\beta$  chain of the most expanded CD8 TCR, including the CDR regions, in a particular HLA haplotype, we aim to reconstitute it in vitro in order to characterize mimotopes recognized by this TCR clonotype. If successful, this very ambitious research strategy may, at long last, permit a causal therapy of AA by specifically targeting pathogenic CD8+ T cells and/or by tolerizing patients against the identified (auto-)antigens.

## P078 | The role of dermoscopy in the evaluation of inflammatory dermatoses

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The use of dermoscopy for evaluation of skin neoplasms is wellestablished. Less is known about the utility of dermoscopy for evaluation of inflammatory skin conditions. We performed a systematic review of the English literature to identify reports of dermoscopy and inflammatory skin disease. One hundred and forty-six inflammatory skin conditions were searched in combination with dermoscopy or dermatoscopy or epiluminescence microscopy. Three hundred and sixteen articles met inclusion criteria. Psoriasis (32), lichen planus (27), lupus (27), scleroderma (18), and dermatitis (17) were the inflammatory conditions most reported with respect to dermoscopy. Based on these results, many inflammatory dermatoses are amenable to evaluation with dermoscopy. Knowledge of the dermatoscopic findings of inflammatory dermatoses may be valuable to clinicians, especially those with special interest in immune mediated skin disease.

#### P079 | Efficacy of an emollient containing Rhealba oat plantlets on idiopathic pruritus among elderly French outpatients

J. Theunis; A. B. Rossi; <u>N. C. Rizzi</u>; V. Mengeaud Pierre Fabre Dermocosmetique, Toulouse, France The prevalence of pruritus is high among the elderly population, but surprisingly the effect of an emollient on pruritus has been little studied in this population.

This controlled, randomized clinical study was performed to assess the efficacy and the safety of an emollient on pruritus and xerosis among French elderly outpatients.

Thirty patients aged 75.8 $\pm$ 8.26 years suffering from chronic idiopathic pruritus with intensity  $\geq$ 5 on a visual analog scale (VAS 0-10) and xerosis  $\geq$ 2 on the Overall Dry Skin Score (0-4) were enrolled.

Subjects were randomized in two cross-over groups so that half patients began with an untreated period of 2 weeks (without application of any moisturizing product) followed by a treated period of 2 weeks during which patients applied the tested emollient once or twice daily. The other half of patients began with a treated period of 2 weeks followed by a wash-out period of 2 weeks in order to go back to a sufficient level of pruritus and xerosis and they ended by an untreated period of 2 weeks. Pruritus was evaluated through VAS and xerosis severity was assessed on a 0-4 scale.

Immediately after the first emollient application, pruritus intensity was significantly decreased as measured by VAS (6.24 vs 3.96; *P*<.0001): 80% of the patients experienced an improvement of pruritus intensity. From 7 days of emollient application, pruritus intensity was significantly decreased compared to untreated period as measured by VAS (*P*=.0002). This efficacy persisted until 4 weeks after the last application (-67.2%) in the group having begun with treated period.

Xerosis was significantly decreased during treated period compared to untreated period as assessed by the overall dry skin score after 1 and 2 weeks (P=.0002 and P<.0001).

This study demonstrated the good tolerance and the efficacy of this emollient reducing pruritus intensity from the first application and reducing xerosis among elderly people suffering from idiopathic chronic pruritus.

## P080 | Evaluation of serum uric acid among patients with psoriasis in developing country

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**Background:** Psoriasis is a common chronic inflammatory skin disorder, Studies have showed that psoriasis can progress to systemic involvement like psoriatic arthritis, metabolic syndrome, uric acid and lipid metabolism derangement. The aim of this study was to evaluate serum uric acid level among patients with psoriasis.

**Method:** From the department of dermatology of two tertiary-care hospital in Kathmandu. 138 patients were enrolled in the study. Among them male=36, female=33 with psoriasis were selected as case, 69 patients with other dermatologic diseases after matching age and sex were selected as controls. Informed written consent, after a detail history, physical examination, BMI and PASI of cases was calculated and sent for serum uric acid investigation. The serum uric acid level was determined for both case, control.

**Result:** Chronic plaque psoriasis was the most common 60(87.0%) variant and there was no significant association between psoriasis type and sex. Male female ratio was 1.09:1. Majority of the psoriasis patients (76%) were among the younger population and most of them (91.3%) had normal serum uric acid level. Most of the patients 55(79.7%) did not have family history of the psoriasis. Patients 58(84.1%) had history of flare up in winter season. BMI was found to be normal range in most 65(94.2%) of the patients. Among control group, eczema was the most common 16(23%) diagnosis and most of the patients (91.3%) had normal serum uric acid level. No significant association between psoriasis area severity index score and serum uric acid level was found in the study (P=.81).

**Conclusion:** In our study, serum uric acid was significant among psoriasis patients than control group. Most of the patients had aggravating psoriasis in winter season. However, it was not also significant with psoriasis area severity index, this may be due to psoriasis lesions were not severe or extensive body surface area involvement and no systemic complication issues. However, we have to rule out other systemic complications due to psoriasis in long term follow up.

### P081 | Occupational eczema and how the community responses in developing country

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**Background:** Developing country like Nepal has still doing occupational works without gloves. Allergic contact dermatitis is one of the important occupational hazards in workers and it often leads to poor quality of life of the workers with substantial financial loss. However, this is often a neglected entity.

**Objective:** This study has been done to assess the allergological profile among the workers in various fields.

Materials and Methods: The study was conducted among the workers working on construction and brick industry. Ninety six workers were selected on clinical suspicion. forty three were selected randomly and patch tested with patch test allergens. Analysis of reactions and relevance of positive test was assessed as per standard guidelines.

**Results:** Both the workers were men and women. Average age of workers was 24.8 years (range, 19-34 years). Eczema affected exposed parts in 93.75% and covered areas in 62.5%. Most common allergens were chromate (relevant allergy/RA: in 60% of patch tested workers), epoxy resin (RA: 30%), cobalt (RA: 20%), nickel (RA: 20%), thiuram mixture (RA: 10%) and black rubber mix (RA: 10%) and few have hair dye. 20% had irritant contact dermatitis.

**Conclusion:** The result indicated that chromate is the most frequent allergen among construction workers. High frequency of involvement of the covered areas as well as the exposed areas highlighted the fact that the allergens had access to most body parts of the workers.

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#### P082 | Epicutaneous treatment with a p38 MAPK activator is sufficient to induce the IL-17-dependent psoriasis-like dermatitis in a new animal model

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Experimental and clinical evidence has suggested that the activation of the IL-23-IL-17 axis is essential in the development of psoriasis, which is considered to be initiated by mediators from the epidermis. However, a molecular cue that specifically induces the activation of this axis in response to the epidermal stresses remains to be clarified, and there are very few systems to explore the cue despite a number of existing animal models. It is of note that the activation of p38 in the epidermal keratinocytes was reported in psoriasis and its animal models. We therefore hypothesized that the activation of p38 in the epidermis, in response to various stresses, is a primary cue for the activation of the IL-23-IL-17 axis and the subsequent development of psoriasis. First we found that the treatment with a chemical p38 activator, anisomycin, rapidly induces the expression of psoriasis-related mediators, including IL-1a and β, IL-6, CXCL1, and IL-24, in mouse or human keratinocytes in vitro. We next tested whether epidermal activation of p38 specifically develops psoriasis-like dermatitis in animals. Strikingly, daily topical treatment with anisomycin induced scaly erythema with acanthosis in mouse ears in 5 days, and a dose-dependent increase of the ear thickness. The expression of the IL-23-IL-17 axis genes, such as IL-23p19, IL-17, and IL-22, infiltration of neutrophils, and IL-17A-producing T cells, all of which are a key signature in psoriasis, were significantly induced in the anisomycin-treated skin. Furthermore, we found a reduced response to anisomycin in mice lacking IL-17, suggesting a critical role of IL-17 in this animal model as it does in psoriasis. Moreover, the treatment with a p38a-specific inhibitor, BIRB796, attenuated all these responses in vitro and in vivo. Taken together, our work suggests that the activation of p38 in the epidermis is a molecular cue to activate the IL-23-IL-17 axis and sufficient to induce psoriatic dermatitis. We thus propose that epidermal p38 is a possible target for the treatment of psoriasis.

#### P083 | CD34+ skin resident precursor cells may contribute to maintain tissue resident macrophages in human skin and are stimulated by substance P and IFNγ

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Besides monocyte-derived macrophages (MACs), self-renewing tissue-resident macrophages (trMAC), are now appreciated to

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Keywords: Cement, chromate, construction workers.

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maintain the "physiological" number of intra-cutaneous MACs in murine skin. However, it remains unknown whether such self-renewing trMAC also exist in human skin and how they may be stimulated. We have investigated this possibility in organ-cultured full-thickness human skin, using the pro-inflammatory neuropeptide, substance P (SP), to imitate a "neurogenic skin inflammation" signaling milieu. In the absence of perfused vasculature or bone marrow or intraluminal CD14+ monocytes. SP significantly increased the number of CD68+ MAC in human dermis ex vivo. Moreover, SP did not suppress MAC apoptosis, as shown by CD68/TUNEL double-immunostaining, and, almost no proliferative CD68+ cells were detectable in test or control skin. This not only suggested that new MACs can arise from resident cells within human skin, but also that proliferation of trMAC is very unlikely to generate the new CD68+ MAC. Interestingly CD34+ cells, MAC precursor cells (but not c-Kit+ cells), were often found to be in direct cell-cell-contact with CD68+ cells in human skin, especially in SP-treated samples. Therefore, we are currently probing the hypothesis that human skin trMACs arise from tissue-resident CD34+ cells. Similar phenomena were also seen in cytokine-mediated inflammation, triggered by IFNy in our full thickness skin organ culture, suggesting that resident MAC progenitor cells exist in human skin and are able to respond to different inflammatory stimuli to satisfy the demand of MAC, notably under conditions of skin stress (such as skin organ culture).

## P084 | Contribution of IgE autoantibodies to the pathogenesis of bullous pemphigoid

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Bullous pemphigoid (BP) is an auto-immune blistering disease that has consistently been associated with IgG autoantibodies and complement activation. Two keratinocyte-produced antigens are known to be the target of IgG auto-antibodies in this disease, ie BP230 (BPAg1) and BP180 (BPAg2). The NC16A portion of the BP180 molecule is thought to have the greatest antigenicity. The frequent appearance of urticarial plaques in a large number of these patients points to a pathogenic role of IgE, but the exact mechanisms of such an occurrence are only poorly understood. In this study, we have addressed this question and have detected, via ELISA, significantly higher levels of NC16A-(P<.0001) and BP230-specific (P<.005) IgE in BP sera than in those of healthy individuals. Using overlapping peptides of BP180 as targets, IgG and IgE were found to share the same dominant epitopes. This observation is consistent with a class-switch phenomenon. IgE was also detected in perilesional skin of 21 out of 32 (66%) BP patients. As opposed to reports from other investigators, this IgE was not found at the dermal-epidermal junction, but rather on the surface of mast cells and eosinophils. We have evidence that, on both cell types, the high-affinity receptor for IgE (FccRI) is the primary molecule involved in this interaction. Our further finding of BP180 co-localizing with IgE+

eosinophils suggests that this antigen (or fragments thereof), released from basal keratinocytes, may cross-link eosinophil- and perhaps also mast cell-bound BP180-specific IgE and induce signal transduction, resulting in mediator release (eg histamine) and manifesting clinically as urticarial tissue inflammation. We therefore propose a new pathway of BP pathogenesis, not alternative but parallel to an IgG track, dependent on functional BP180-IgE immune complexes present in BP skin.

#### P085 | Induction of alternative proinflammatory cytokines accounts for sustained psoriasiform skin inflammation in IL-17C+IL-6KO mice

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IL-6 inhibition has been unsuccessful in treating psoriasis, despite high levels of tissue and serum IL-6 in patients. Additionally, de novo psoriasis onset has been reported following IL-6 blockade in rheumatoid arthritis patients. To explore the mechanisms underlying these observations, we backcrossed an established psoriasiform mouse model (IL-17C+ mice) with IL-6 deficient mice (IL-17C+KO). IL-17C+KO mice initially exhibit decreased skin inflammation, however this decrease was transient and reversed rapidly, concomitant with increases in skin Tnfa, II36 $\alpha/\beta/\gamma$ , II24, and S100a8/a9 to levels higher than those found in IL-17C+ mice. Comparison of the cellular transcriptome in lesional IL-17C+ and IL-17C KO mouse skin by RNAseq against human psoriasis revealed significant correlation between psoriasis patient skin and IL-17C+KO mouse skin and confirmed an exacerbation in the inflammatory signature in IL-17C+KO mice that aligns closely with human psoriasis. Transcript analyses of primary keratinocytes grown from IL-17C+ and IL-17C+KO mice confirmed the increased expression of proinflammatory molecules, suggesting that in the absence of IL-6, keratinocytes increase production of numerous additional proinflammatory cytokines. These preclinical findings may provide insight into why arthritis patients being treated with IL-6 inhibitors develop new onset psoriasis and why IL-6 blockade for the treatment of psoriasis has not been clinically effective.

#### P086 | Imiquimod has strain-dependent effects in mice and does not uniquely model human psoriasis

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Imiquimod (IMQ) produces a cutaneous phenotype in mice frequently studied as a model of acute psoriasis. Whether this phenotype depends on strain or sex, however, has seldom been addressed. This study used RNA-seg to evaluate the psoriasiform phenotype elicited by IMQ in both sexes of 7 mouse strains (C57BL/6J, BALB/cJ, CD1, DBA/1J, FVB/NJ, 129X1/SvJ and MOLF/EiJ). In most strains, IMO altered gene expression in a manner consistent with human psoriasis. Key aspects of the IMO response, however, differed significantly between strains. Surprisingly, although IMQ-induced expression shifts mirrored psoriasis, correspondence was similar or better for other human skin diseases (eg eschars, acne, infection, wounding). These findings demonstrate strain-dependent aspects of IMQ dermatitis that warrant consideration in planning and interpreting experimental studies. We have further shown that the IMQ phenotype does not uniquely model psoriasis but in fact triggers a core set of pathways active in diverse skin diseases.

### P087 | Novel chimeric immunoreceptors for pemphigus vulgaris (PV) therapy

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Pemphigus therapy relies on chronic immunosuppression, which causes significant morbidity and mortality. Ideally, therapy should target pathogenic autoimmune cells while sparing protective immunity. Alternatively, complete, transient B cell depletion should cure PV, since autoreactive clones do not recur upon generation of a new B cell repertoire. To achieve these goals, we investigated 2 immunotherapy strategies. For targeted therapy, we designed chimeric autoantibody receptors (CAARs), consisting of the extracellular domains of the PV autoantigen, desmoglein 3 (Dsg3), fused to T cell cytoplasmic signaling domains. CAARs direct human T cells to specifically kill B cells expressing surface anti-Dsg3 IgG both in vitro and in vivo in a PV mouse model. Circulating anti-Dsg3 IgG do not compromise CAAR-T cell function, as mAbs with slower offrates reduce but still preserve significant CAAR-T cytotoxicity. To investigate safety in vivo, we injected CAAR-T cells in human skin xenografted mice. CAARs cause no epidermal toxicity compared to an anti-CD19 T cells. These data indicate that Dsg3 CAAR-T cells are specific and effective in targeting autoimmune B cells in PV. For complete, transient B cell depletion, we designed an anti-CD19 chimeric antigen receptor, co-expressed with an inducible caspase-9 "suicide" gene (sCAR). sCAR-T cells effectively deplete CD19+ Nalm6 B cells in an in vivo mouse model as well as in fully humanized BLT mice. Induced activation of caspase-9 results in efficient in vivo depletion of sCAR-T cells, showing the feasibility of this strategy. In summary, we have validated 2 novel, non-redundant approaches for immunotherapy of PV, which can be applied to other autoimmune diseases.

## P088 | Biosimilar Infliximab: Is intracellular signaling the same after switching?

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Psoriasis is a chronic, inflammatory skin disease characterized by a dysregulated immune response. Biological treatment against TNFa has been shown to be beneficial. However, even though this treatment is effective, it is very expensive and some patients experience serious adverse events. Biosimilars are cheaper generic versions, but it remains to be seen if they are comparable when it comes to efficacy and safety. We here compare signaling responses in peripheral blood mononuclear cells of psoriasis patients treated with the innovator infliximab (Remicade<sup>®</sup>) and the biosimilar infliximab (Remsima<sup>®</sup>). We used phospho-specific flow cytometry to investigate possible differences in cellular responses against the original drug and the biosimilar. 25 patients with psoriasis and 19 healthy controls were included. The signaling signatures will be analyzed regarding phosphorylation of ERK, p38, NF-KB, and STAT3 and compared with clinical parameters. The results will give important insights into usage of biosimilars and the discussion of using them as interchangeable drugs.

#### P089 | Calcipotriol enhances cathelicidin expression and improves bacterial defense and wound closure in epidermolysis bullosa

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Recessive dystrophic epidermolysis bullosa (RDEB) is a rare genetic skin disorder characterized by blister formation between the epidermis and the underlying dermis. Patients with RDEB suffer from chronic open wounds which are susceptible to microbial infections that further delay wound healing and promote ongoing inflammation. An often overlooked factor that is critical for wound healing is vitamin D3 (VitD3). Importantly, keratinocytes are unique in that they possess the entire enzymatic machinery required to produce active -WILEY—Experimental Dermatology

VitD3. Skin injury enhances VitD3 production and triggers the expression of VitD3-target genes needed for wound healing, including the antimicrobial peptide cathelicidin (hCAP18). Thus, enhancing active VitD3 levels at sites of injury may prove beneficial to RDEB patients. Interestingly, we found that basal levels of hCAP18 were significantly lower in 3 out of 5 RDEB cell lines compared to normal human keratinocytes, suggesting a possible VitD3-deficiency in at least a subset of RDEB. To provide initial evidence of a potential benefit of treatment with the VitD3 analog calcipotriol, we investigated the responsiveness of these RDEB cell lines to the drug using induction of hCAP18 as readout. We confirmed a dose dependent induction of hCAP18 on both mRNA and protein level. The effect of calcipotriol treatment on cell proliferation and wound closure was further investigated using MTT and scratch assays. Calcipotriol had a small positive impact on RDEB keratinocyte proliferation at concentrations up to 100 nmol/L, indicating an effective threshold concentration for treatment. Scratch assays demonstrated reduced wound closure of RDEB keratinocyte compared to normal keratinocytes that could be enhanced up to 2-fold following treatment with 100 nmol/L calcipotriol. Finally, antimicrobial assays showed a reduction in viability of C. albicans, a common wound colonizer in EB, when incubated in cell culture supernatants harvested from calcipotriol-treated compared to untreated RDEB keratinocytes. Taken together, our data showed that calcipotriol treatment results in improved wound closure rates and enhanced antimicrobial defense in vitro, suggesting a potential clinical benefit for RDEB patients.

#### P090 | Association between patient-perceived effectiveness of immunosuppressants/ phototherapy and patient-reported outcomes among adults with atopic dermatitis: Data from a US cross-sectional study (AWARE)

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Objective: To evaluate the relationship between patients' perceptions of the effectiveness of current treatments and patient-reported outcomes among adults with moderate-to-severe atopic dermatitis (AD). Methods: Adults (≥18 years) with moderate-to-severe AD (Rajka-Langeland criteria) were identified from the cross-sectional AWARE (Adults With Atopic dermatitis Reporting on their Experience) study conducted between Jan-1-2013 and Dec-31-2014 at six US academic medical centers. Patients reported on their perception of the effectiveness of current treatment and outcomes, including: itch (pruritus numerical rating scale [NRS]), pain (NRS), sleep (PO-SCORAD visual analog scale [VAS]), and quality of life (Dermatology Life Quality Index [DLQI]).

**Results:** A total of 536 moderate-to-severe AD patients (mean age, 44.8 years; 65.0% female) were treated with immunosuppressants/ phototherapy (IM/PT; 38.2%) or topicals-only (corticosteroids or calcineurin inhibitors; 61.8%) in the past 7 days, of whom 50.2% and 41.4%, respectively, perceived their current treatment to be ineffective. Patients who perceived IM/PT to be ineffective reported significantly worse itch, pain, and sleep than patients who perceived IM/PT to be effective. However, patients who reported IM/PT to be effective had similar patient-reported burden to patients who reported topicals to be ineffective across all outcomes. Mean (SD) scores for the IM/PT-ineffective, IM/PT-effective, and topicals-ineffective groups: pruritus NRS, 6.9 (2.4) vs 4.9 (2.8) vs 5.4 (2.8); pain NRS, 4.7 (3.1) vs 2.6 (2.7) vs 2.7 (2.6); sleep VAS, 4.7 (3.4) vs 3.1 (3.2) vs 3.9 (3.1); DLQI, 13.3 (6.9) vs 8.0 (6.3) vs 8.7 (6.3), respectively.

**Conclusions:** Half of moderate-to-severe AD patients receiving IM/ PT perceive these treatments to be ineffective and report a high burden of disease. Patients who perceive IM/PT to be effective report similar itch, pain, sleep loss, and impaired QoL as patients who perceive their topical treatments to be ineffective. These data indicate that expectations of benefit from existing treatments are low among adults with moderate-to-severe AD, suggesting a need for more effective treatments for these patients.

#### P091 | Epigenetic modifications in skin-homing CD4+CLA+ T-cells of atopic dermatitis patients relate to mRNA expression changes in high mobility group proteins

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**Background:** T cells expressing the cutaneous lymphocyte antigen (CLA) mediate pathogenic inflammation in atopic dermatitis (AD) but the molecular alterations contributing to their dysregulation remain unclear.

**Objective:** We profiled differences in DNA methylation, miRNA and mRNA expression in sorted T cells from AD patients and healthy controls (HC) aiming to elucidate putative altered pathways in AD.

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**Methods:** Twenty age- and sex-paired adult AD patients and HC were included. Four T cell populations (CD4+, CD4+CD45RA+ naïve, CD4+CLA+, and CD8+) were isolated from peripheral blood by magnetic sorting. Then a multilayered, integrative analysis of genome-wide DNA methylation, (Illumina HumanMethylation450BeadChip), miRNA expression (Agilent 8x60K-array) and mRNA transcript levels was performed.

**Results:** Skin homing CD4+CLA+ T cells from AD patients showed significant differences in DNA methylation levels compared to HC at 49 CpG-sites. Genes harboring these CpG-sites are involved in intracellular signal transduction and cytokine signaling. One of the CpG sites lies within the upstream region of the interleukin 13 gene (IL13), and methylation levels correlated with increased IL13 mRNA expression in the CD4+CLA+ T cells from the patients. Moreover, 16 miRNAs showed differential expression in CD4+CLA+ T cells from AD patients compared to HC (10 up-regulated and 6 down-regulated). mRNA levels for the high mobility group (HMG) proteins HMGB2 and HMGN2 were up-regulated in AD patients compared to HC and were predicted targets of the differentially expressed miRNAs.

**Conclusions:** Using data integration putative altered molecular pathways in circulating CD4+CLA+ T cells from patients with AD were discovered. HMG proteins may promote pro-inflammatory functions in CD4+CLA+ T cells in AD patients and thereby contribute to the pathogenesis of the disease. Genes encoding HMG proteins are emerging novel candidates for prevention and therapeutics of AD.

# P092 | CD49a expression reveal functional dichotomy in cytokine production and cytotoxic capacity in tissue resident T cells in healthy, psoriasis and vitiligo affected skin

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Tissue resident memory T cells (Trm) provide immediate defence against recurrent infections in epithelial barriers. Here, we identify a population of epidermal CD8+ Trm cells in healthy human skin marked by the surface expression of CD49a. Transcriptome analyses revealed preferential expression of cytotoxicity related genes in CD49a+ Trm, albeit negligible protein expression of perforin and granzyme B. Consequently, CD49a+ Trm displayed limited cytotoxic function during homeostasis. Rapid up-regulation of perforin and granzyme B expression was induced by IL-15 or IL-2 stimulation which unleashed strong cytotoxic capacity in CD49a+ but not in CD49a- Trm. In vitiligo, an autoimmune disease characterised by aberrant killing of melanocytes, perforin and granzyme B expressing CD49a+ Trm were highly enriched in both dermis and epidermis. Conversely, CD49a- Trm cells displayed augmented IL-17 responses in psoriasis, where this cytokine promotes skin inflammation. Together, our results delineate compartmental and functional specialization among CD8+ Trm cells in human epidermis that is preserved in two distinct inflammatory diseases.

#### P093 | Efficacy, safety and pharmacodynamics of a high-affinity anti-IgE antibody in patients with moderate to severe atopic dermatitis: A randomized, double-blind, placebo-controlled, proof-of-concept study

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Atopic dermatitis (AD) is a therapeutically challenging chronic skin disease. The majority of AD patients exhibit increased total serum IgE values accompanied by sensitization to various allergens with yet unclear relevance to the eczematous response. Recent trials using the monoclonal anti-IgE antibody omalizumab did not show clinical efficacy despite a decrease of free serum IgE and its corresponding receptor FcERI on leukocytes. Thus, an anti-IgE antibody with higher affinity than omalizumab, QGE031, was tested to assess safety and efficacy in adult AD patients Adult patients with moderate to severe AD were treated in a double-blind fashion every 2 weeks, for a total of 12 weeks, with either SC administration of QGE031 280 mg (n=10) or placebo (n=10). Skin biopsies obtained at baseline and after 12 weeks of treatment were immunohistologically evaluated for the amount of IgE and FcERI on leukocytes. In addition, clinical disease scores, drug safety and IgE-related biomarkers were assessed.

Anti-IgE treatment was not significantly superior to placebo in inducing an EASI-50 clinical response at week 12. Likewise no significant difference was observed for either pruritus or sleep disturbance. A complete suppression of circulating IgE was only achieved in patients with baseline total IgE levels<1800 IU/mL. Similarly, skin biopsies evaluated for the amount of IgE and FcERI present on mast cells and dendritic cells confirmed that patients with initial higher IgE concentrations did not achieve the same levels of suppression as patients with lower baseline IgE levels. Reduced IgE reactivity was functionally confirmed by a clear downregulation of allergic skin prick tests in the treatment arm. Overall the study drug was well tolerated with a safety profile similar to placebo. In summary, this trial failed to demonstrate clinical efficacy of a 12 week treatment with a high affinity anti-IgE antibody in adult AD patients. These findings confirm the only limited impact of IgE-mediated processes on the pathogenesis of the eczematous response in AD.

#### P094 | $\beta$ -defensin-2 (BD-2) responds to a single dose of anti-IL-17A secukinumab in different skin layers of psoriatic patients within days

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Abstracts

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Secukinumab, a fully human monoclonal antibody selectively targeting IL-17A, is highly efficacious in the treatment of moderate to severe psoriasis, psoriatic arthritis and ankylosing spondylitis. We and others have shown that IL-17A is increased in psoriatic lesions and that BD-2 decreases significantly and rapidly in serum samples. BD-2 is a keratinocyte derived antimicrobial peptide that can be stimulated by the combination of TNF $\alpha$  and IL-17A. In a small pilot study in eight healthy volunteers (HV) and eight psoriasis patients, we examined BD-2 in serum samples as well as in dermal fluid samples from open flow microperfusion, and in epidermal samples from the upper stratum corneum (tape strips).

BD-2 protein levels in serum were correlated to the clinical severity (PASI) and to baseline IL-17A levels, suggesting a strong link in the pathophysiology of psoriasis. Levels of BD-2 in serum were 82 pg/mL at baseline in HV samples and 5746 pg/mL in psoriasis patients, which decreased to levels similar to that of HV after a single subcutaneous dose of 300 mg secukinumab. Skin levels were derived from open flow microperfusion samples and showed that BD-2 levels in HV were 109 pg/mL, slightly higher in non-lesional skin of psoriatic patients (417 pg/mL), but increased to 2747 pg/mL in lesional skin. These levels decreased after single dose of secukinumab to levels close to those in HV. In contrast, mean BD-2 levels in the lesional upper stratum corneum sample were much higher at baseline (35 244 pg/mL), decreasing to a mean of 11 264 pg/mL at 14 days post-dose. As described before, BD-2 is detected mainly in the upper epidermal layers of psoriasis plaques.

BD-2 is a responsive marker for psoriasis pathophysiology and can be sampled and analyzed from blood, dermal fluid or non-invasive stratum corneum samples. In this small trial, secukinumab decreases rapidly BD-2 in serum and the skin compartments.

#### P095 | Inflammatory skin conditions in patients under anticancer therapies: Observations from an oncodermatology clinic

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Anticancer therapies, including radiation, systemic agents, and stem cell transplants may result in dermatologic adverse events (dAE), negatively impacting dosing and quality of life. We report the inflammatory-mediated dAE in patients under anticancer therapies referred to an oncodermatology clinical program and the differences in management between referring oncologist and dermatologist. A retrospective chart review of 130 outpatient dermatology consultations over a two month period (January-February, 2015) was performed. Patients at Memorial Sloan Kettering were identified through a consult log maintained by the dermatology service administrative personnel. Relevant clinical information was abstracted from each patient's Electronic Medical Record. Demographics, reason for referral diagnosis, tumor type, anticancer therapy agents, and dermatologic diagnosis were amongst the variables of interest recorded. A total of 113 patients were included. Sixty-two patients (54.9%) were diagnosed of an inflammatory skin condition, followed by infections (13.3%). Rash was the most common clinical diagnosis in 38 (61.3%), of which a maculopapular rash related to anti-cancer agent was the most frequent (34.2%). Skin biopsies were obtained in 20 patients. Referring clinicians and dermatologists agreed diagnostically only on 26% of the conditions referred and in 77 patients, there was a lack of concordance. Referring clinicians and dermatologists agreed on continuing anti-cancer therapy in 76% of cases ( $\kappa$ =0.14). In summary, this data demonstrates that inflammatory skin conditions are the most common cause of referral for dermatologic evaluation in oncology patients, and underscores the importance of a dermatologist's evaluation in the diagnosis, attribution, and managements of dAEs.

## P096 | Efficacy and safety of ixekizumab in patients previously treated with etanercept

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Ixekizumab (IXE) is a high-affinity monoclonal antibody that selectively targets interleukin-17A. We evaluated the efficacy and safety of 44 weeks of IXE treatment when used after 12 weeks of etanercept (ETN) treatment for psoriasis. Patients were randomized to 12 weeks of subcutaneous placebo (PBO; N=193); ETN 50 mg, twice weekly (N=382); or 80 mg IXE as 1 injection every 2 weeks (IXE Q2W; N=385) or every 4 weeks (IXE Q4W; N=386) following an initial 160-mg starting dose. During the open-label extension period (Weeks 12-60), PBO patients received 160 mg IXE at Week 12, followed by IXE Q4W (Weeks 16-60); ETN patients received 2 injections of PBO at Week 12 (to provide a 4-week washout period), followed by IXE Q4W (Weeks 16-60). Efficacy endpoints were percentages of patients achieving ≥75%, ≥90%, or 100% improvement in Psoriasis Area and Severity Index (PASI 75, 90, or 100). At Week 12, 7.3%, 3.1%, and 0% of patients treated with PBO achieved PASI 75, 90, and 100, respectively; 53.4%, 25.7%, and 7.3% of patients treated with ETN achieved PASI 75, 90, and 100, respectively. At Week 12, 183 patients treated with PBO and 369 patients treated with ETN entered the open-label extension. After 12 weeks of treatment with IXE Q4W, 91.3%, 76.5% and 44.3% of patients previously treated with PBO (PBO/IXE Q4W) and 88.3%, 74.8%, and 46.1% of patients previously treated with ETN (ETN/IXE Q4W) achieved PASI 75, 90, and 100, respectively. By Week 60, 87.4%, 79.2%, and 55.2% of PBO/IXE Q4W patients and 85.4%, 78.3%, and 55.8% of ETN/

IXE Q4W patients achieved PASI 75, 90, and 100, respectively. There were no unexpected safety signals in UNCOVER-3 in patients treated with IXE. Patients previously treated with ETN achieved a high level of skin clearance and maintained improvements over 44 weeks after being switched to open-label IXE treatment, and experienced a similar safety profile when compared with those who were switched from PBO to IXE.

## P097 | Efficacy and safety of ixekizumab over four years of open-label treatment in a Phase 2 study in chronic plaque psoriasis

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Ixekizumab (IXE), a high-affinity monoclonal antibody that selectively targets interleukin-17A, has exhibited efficacy in resolving psoriatic lesions in Phase 2 and Phase 3 trials. We evaluated efficacy and safety in patients treated with IXE for at least 4 years. After completing a 20week randomized treatment period (RTP), patients were eligible to enter the open-label extension (OLE) of the Phase 2 trial, dependent on clinical response. All patients entering the OLE were treated with 120 mg IXE every 4 weeks (Q4W) through Week 88. From Weeks 92 to 124, all patients were switched to 80 mg Q4W for the remainder of the study. Efficacy data are presented as observed or imputed using last observation carried forward method. Safety was evaluated up to and past 208 weeks (4 years) of treatment in the OLE. The yearto-year incidence rates of adverse events (AEs) were reported per 100 person-years. Of the 129 patients who completed the RTP, 120 entered the OLE, and 75 (62.5%) completed at least 4 years of treatment. Of the 47 patients who discontinued during the OLE (up to and past 4 years), 11 were due to treatment-emergent AEs (TEAEs) and 10 were reported as lack of efficacy. Clinical response rates (PASI 75, 90, and 100) were stable throughout the OLE. The exposureadjusted year-to-year incidence rates for all TEAEs and serious AEs declined over time. Most TEAEs were considered mild or moderate. The most common TEAEs (≥10% patients experiencing AEs) were nasopharyngitis (23.3%), sinusitis (13.3%), upper respiratory tract infection (12.5%), and headache (10.0%). Seven patients reported candida infections, one patient reported two major adverse cardiac events, and seven patients reported a malignancy (3 were non-melanoma skin cancer). No severe neutropenia (grade 3 or 4 neutropenia) was reported.

In patients enrolled in an OLE with IXE Q4W, response to treatment was maintained in the majority over 4 years. Safety signals observed up to and beyond 4 years were consistent with those previously reported.

#### P098 | Time course of ixekizumab drug levels and the relationship at week 60 to efficacy in patients with moderate-to-severe plaque psoriasis (UNCOVER-3)

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Ixekizumab (Taltz<sup>®</sup>), a high-affinity monoclonal antibody that selectively targets interleukin-17A, has recently been approved in the United States and European Union for patients with moderateto-severe plaque psoriasis. The time course of ixekizumab serum drug levels and the relationship with key efficacy endpoints (static Physician Global Assessment [sPGA] and Psoriasis Area and Severity Index [PASI]) after 60 weeks of dosing, using data from a Phase 3 study (UNCOVER-3) are described. Ixekizumab was administered subcutaneously as a 160-mg initial dose (Week 0), then an induction dose of 80 mg every 2 weeks (Q2W) or every 4 weeks (Q4W) to Week 12 (Period 2). Patients then entered the long-term extension period [Week 12 (Visit 7) up to Week 264 (Visit 36)] to evaluate the safety and efficacy of 80 mg ixekizumab Q4W (Period 3). Data for this analysis were available up to Week 60. Pharmacokinetic samples were taken at the following time points post-dosing in all patients-4, 12, 24, 36, 48, and 60 weeks-and were summarized over time. Efficacy endpoints at Week 60 were sPGA score 0/1 or 0 and PASI (at least a 75%, 90%, or 100% improvement from baseline in PASI score [PASI 75/90/100]) and were summarized by concentration quartile. The data set included 373 patients receiving the Q2W/Q4W dosing regimen and 371 receiving the Q4W/Q4W dosing regimen. At Week 24 (when all patients were on Q4W maintenance dosing), mean serum drug levels for each dosing group were similar (mean concentrations ranged from 3.39 to 3.66 µg/mL), and remained consistent from Week 24 through Week 60 of the study, across both cohorts. Higher Week 60 response rates were generally observed for higher serum drug levels. This was most apparent for PASI 100 and sPGA 0. Up to Week 60, PASI 100 and sPGA 0 response rates were higher for Q2W/Q4W. Long-term serum ixekizumab levels were stable over time and associated with high rates of disease clearance.

## P099 | Attenuated dynamic of IMQ-induced psoriasis in inflammasomes KO mice models

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Psoriasis is one of the most common chronic inflammatory skin diseases. Cross-talk between activated keratinocytes, innate and adaptive immune systems are considered to be important for its -WILEY—Experimental Dermatology

pathogenesis. We have used an IMQ-inducible mouse model to study the role of inflammasomes, intracellular immune complexes sensing danger- or pathogen-associated molecular patterns, in triggering of psoriasiform skin inflammation. WT as well as three inflammasomes KO mice strains (NLRP3 KO, ASC KO and NLRP12 KO mice on C57 BI/6 background) have been treated by control, placebo and Aldara for 6 consecutive days. Placebo treatment alone doesn't lead to psoriasis-like dermatitis in any of the studied genotypes. Aldara treatment induces psoriasis-like dermatitis in all genotypes, but with different dynamics and intensity. NLRP3 and ASC KO mice had delayed responses and while, comparing to WT, show the similar pattern of skin hyperkeratosis and acanthosis and systemic immune responses, had significantly less infiltration of immune cells in dermis and epidermis. Interestingly, NLRP3 KO mice show different dynamics of TEWL upon IMQ treatment, indicating the possible inflammasome independent role of NLRP3 in barrier function. While NLRP3 and ACS KO mice show delayed dynamic of psoriasis induction, NLRP12 KO mice were phenotypically much more affected after 6 days of treatment indicating a possible protective role of NLRP12 protein in induction of psoriasis.

## P100 | The inpatient burden of pediatric autoimmune blistering disease in the United States

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**Background:** Little is known about the epidemiology of pediatric autoimmune blistering disorders (AIBD). We sought to determine the inpatient burden and comorbidities of pediatric AIBD.

**Methods:** Cross-sectional study of the Nationwide Inpatient Sample from 2002 to 2012, containing a representative 20% sample of all US hospitalizations.

**Results:** The most common AIBD with a primary admission was pemphigus (8.0 per million), whereas the most common secondary diagnosis of AIBD was dermatitis herpetiformis (DH; 9.6 per million). Bullous pemphigoid (BP) was inversely associated with being female and government or no insurance, but positively associated with black and Hispanic race/ethnicity and more chronic conditions. Pemphigus was associated with being female, Hispanic, government or no insurance and higher number of chronic conditions. DH was inversely associated with non-white race, but positively associated with government insurance and increased chronic conditions. BP was associated with dialysis, hypertension and diabetes. Pemphigus was associated with osteoarthritis, renal failure, hypothyroidism and weight loss. DH was associated with herpes simplex virus infection, rheumatoid arthritis, fungal, viral and other skin infections.

**Conclusions:** There was a substantial inpatient burden and morbidity of pediatric AIBD.

## P101 | Psoriasis and psoriatic arthritis are associated with osteoporosis and pathological fractures

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**Background:** Psoriasis is a chronic inflammatory skin disease associated with significant morbidity. We sought to determine whether psoriasis (PsO) and psoriatic arthritis (PsA) are associated with osteoporosis and fractures in US adults.

**Methods:** The Nationwide Emergency Department Sample from 2006 to 2012 containing 20% of all US emergency department visits was analyzed. ICD-9-CM codes were used to identify PsO (n=183 725) and PsA (n=28 765).

**Results:** In pooled analysis across all 7 years, patients with PsO had significantly higher odds of osteopenia (multivariate logistic regression; odds ratio [95% confidence intervals]: 2.86 [2.70-3.02]), osteoporosis (2.97 [2.89-3.06]), osteomalacia (4.40 [2.50-7.74]), ankylosing spondylitis (13.34 [12.02-14.81]), and pathological fractures (2.35 [2.19-2.53]). Similar associations were observed for PsA. PsO was also associated with vertebral (1.17 [1.09-1.25]), pelvic (1.18 [1.06-1.31]), femoral (1.68 [1.60-1.78]) and tibial/fibular fractures (1.28 [1.16-1.41]). Whereas, PsA was associated with stress (2.87 [1.08-7.64]), vertebral (1.45 [1.24-1.70]), pelvic (1.75 [1.41-2.18]), femoral (2.07 [1.85-2.32]) and tibial/fibular (1.60 [1.28-2.01]) fractures.

**Conclusion:** PsO and PsA were associated with osteopenia, osteoporosis, ankylosing spondylitis and pathologic fractures.

## P102 | Epidemiology of erythema multiforme major, Stevens-Johnson syndrome and toxic epidermal necrolysis in US children

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**Background:** Erythema multiforme major (EMM), Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are life-threatening disorders associated with significant morbidity and mortality. Little is known about the epidemiology of EMM/SJS/TEN in children. We sought to determine the morbidity, mortality, and comorbid health conditions of EMM/SJS and TEN in US children.

**Methods:** This was a cross-sectional study of the 2009-2012 Nationwide Inpatient Sample (NIS), which contains a representative 20% sample of all hospitalizations in the US. Socio-demographics, inflation-adjusted cost, length of stay (LOS), comorbidities and mortality were analyzed using descriptive statistics and multivariate regression analysis.

**Results:** The incidences of EMM/SJS, SJS-TEN and TEN were a mean 5.7, 0.8, and 0.4 cases per million children per year, respectively. Prolonged LOS and higher costs of care (EMM/SJS: 9.3±0.6

days, \$24 776±\$3183; SJS-TEN: 15.7±1.5 days, \$63 787±8014; TEN: 20.4±6.3 days, \$102 243±37 588) were observed compared to all other admissions (4.6±0.1 days, \$10 496±424). Mean sex and age-adjusted mortality was 0% for EMM/SJS, 3.7% [1.4-6.1] for SJS-TEN and 24.8% [11.8-37.8] for TEN. In regression models, predictors of mortality included renal failure (311.37 [50.31 to >999.99]), malignancy (56.13 [9.69-325.24]), septicemia (30.77 [7.97-118.75]), bacterial infection (21.07 [5.64-78.70]), and epilepsy (58.07 [11.92-282.77]). **Conclusions:** Pediatric SJS/TEN pose a substantial health burden in the US. BSA >30%, renal failure, septicemia, bacterial infections, and epilepsy were the strongest predictors of mortality. Future studies are needed to improve prognostication and reduce the incidence and mortality of SJS/TEN.

#### P103 | Eczema, atopic dermatitis or atopic eczema? An analysis of lay terms by global search engine trends

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**Background:** The lack of standardized nomenclature for atopic dermatitis (AD) creates challenges for the harmonization of terminology. This creates unnecessary confusion and potentially impacts communication of research. We sought to determine the relative popularity of the terms eczema, AD and atopic eczema (AE) using global search engine volumes.

**Methods:** We performed a retrospective analysis of average monthly search volumes from 2014 to 2016 of Google, Bing/Yahoo and Baidu for eczema, AD and AE in English and 37 other languages. Google Trends was used to determine the relative search popularity of each term from 2006 to 2016 in English and the top foreign languages, German, Turkish, Russian and Japanese.

**Results:** Overall, eczema accounted for 1.5 million monthly searches (84%) compared to 247 000 searches for AD (14%) and 44 000 searches for AE (2%). For English language, eczema accounted for 93% of searches compared to 6% for AD and 1% for AE. Search popularity for eczema increased from 2006 to 2016, but remained stable for AD and AE.

**Conclusion:** Despite its ambiguity, eczema is the most commonly used lay term for AD.

## P104 | The endocannabinoid system: An untapped source for dermatologic therapies

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The endocannabinoid system (ECS) is a family of cell surface receptors, endogenous cannabinoid ligands, and enzymes that contributes -Experimental Dermatology-WILEY

to a myriad of physiological processes spanning from memory and sleep to appetite and metabolism. For millennia, man has appreciated the Cannabis sativa plant for medicinal and recreational purposes. The past three decades have revealed the biological underpinnings for many of the functions of the ECS. Even more recently it has come to light that cannabinoid receptors are expressed widely in the skin, and this has become a burgeoning area of dermatologic research.

Cannabinoid signaling strongly influences the cutaneous inflammatory response. Binding of cannabinoid receptors can redirect T-helper celldirected inflammatory cascades and affects differential expression of key cytokines in various skin diseases. Pain and itch sensation is transmitted, in part, by the ECS. It plays a role in homeostasis of adnexal structures and maturation and proliferation of keratinocytes. ECS tone can be increased or decreased using relatively well-understood cannabinoids, synthetic and "natural". These characteristics mark the ECS as a potential source of therapies aimed at skin diseases such as atopic dermatitis, contact dermatitis, and even skin cancer. Here, we present an overview of the literature as it pertains to the ECS in skin and discuss some of its untapped potential as a tool to treat cutaneous disease.

#### P105 | Altered inflammasome and integrin profiles in circulating classical (CD14+CD16neg) monocytes and adherent monocyte pairs in psoriasis

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Psoriatic individuals have increased circulating inflammatory monocytes, known to play a role in chronic disease and be predictive of cardiovascular disease (CVD), suggesting potential common immune mediator(s) between psoriasis and CVD. We previously reported that individuals with psoriasis exhibit an increase in aggregate cell-cell doublet pairs that include these monocytes in circulation. Furthermore, these doublets can be used as a predictor of psoriasis severity in our test cohort (n=19) when compared to controls (n=23). Consequently, we hypothesized that decreasing inflammatory monocytes and/or adherent monocyte doublet interactions in circulation may decrease CVD-related events observed in psoriasis patients. We performed RNA-Seq (Illumina HiSeq 2500) to determine if the transcriptome of singlet classical monocytes (CD14+CD16neg) differed from the transcriptome of monocyte doublet aggregates isolated by flow-sorting classical monocytes and doublets from psoriasis (n=4) and control (n=8) individuals. Through pathway enrichment analysis, we uncovered significant gene expression (gene t test and pathway enrichment P values<.05) of inflammasome-specific genes, including IL2, chemokine, complement, and IFNG/TGFB signaling pathways, when contrasting singlets and doublets from subjects with or without psoriatic disease. In general, we observed the most significantly upregulated inflammasome expression changes in doublets from -WILEY-Experimental Dermatology

psoriasis patients vs doublets from controls relative to other contrasts (eg doublets vs singlets or psoriasis vs control singlets). Interestingly, ITGB1 and ITGB3 were upregulated across several pathways in psoriasis monocyte doublets, indicating a potential mechanism for their increased adhesion. Moreover, CD9, an integrin-binding member of the tetraspanin family, was increased in both singlet and doublet monocytes of psoriasis patients when compared to controls. Thus inhibition of CD9 may be a promising potential target for preventing the formation of adherent monocyte pairs. Decreasing the number of monocyte aggregates in circulation and therefore effectively disrupting monocyte-monocyte adherence and signaling may decrease the overall inflammatory signature present in the circulation of psoriasis patients thus potentially reducing associated comorbidities, such as CVD.

## P106 | Epidemiological study of pediatric atopic dermatitis severity

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**Background:** Little is known about the predictors of childhood atopic dermatitis (AD) severity. We sought to determine the clinical and epidemiological factors associated with AD severity.

**Methods:** We analyzed data from "A Prospective Pediatric Longitudinal Evaluation to assess the long-term Safety of tacrolimus ointment for the treatment of atopic dermatitis" (APPLES), a phase 4, prospective, multinational, observational cohort study (n=7902). AD severity was determined using the Rajka and Langeland criteria during physical examination by a dermatologist at baseline.

Results: Overall, 355 (4.5%) were clear of AD lesions, whereas 3591 (45.5%) had mild, 3249 (41.2%) has moderate and 699 (8.9%) had severe AD at enrollment. In bivariate analyses, AD severity was inversely associated with AD onset at age 1-5 (ordinal logistic regression; odds ratio [95% confidence interval]: 0.61 [0.56-0.67]), 6-10 (0.57 [0.50-0.66]) and 11-17 (0.50 [0.41-0.61]) compared to<1 year. Subjects with a longer duration of AD were more likely to have more severe disease (1-4 vs<1 years: 1.41 [1.22-1.62]) and severity increased with increasing duration of AD (≥5 years: 2.01 [1.74-2.33]). Asians were at the greatest risk of having more severe disease relative to whites (3.01 [2.48-3.64]), followed by blacks (1.51 [1.37-1.67]), multiracial/ other (1.37 [1.09-1.73]) and Hispanics (1.20 [1.04-1.39]). Those with a history of asthma (1.73 [1.58-1.89], hay fever (1.35 [1.23-1.48]) or other allergies (1.91 [1.75-2.09]) had more severe AD. AD severity was not associated with gender or season of birth. In multivariate regression models using stepwise-selection, age of onset, duration of AD, race/ethnicity and history of asthma and other allergic disease were selected in final models.

**Conclusions:** Older age of onset and prolonged duration of AD and history of atopic disease were associated with increased AD severity. There were significant racial/ethnic disparities with respect to AD severity.

## **P107** | Altered composition of epidermal lipids correlates with *Staphylococcus aureus* colonization status in adult atopic dermatitis subjects

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Atopic dermatitis (AD) is an inflammatory skin condition with wellrecognized sub-phenotypes distinguished by altered skin permeability barrier functions and status of S. aureus colonization. Composition of skin epidermal lipids is altered in AD subjects. Our objective was to determine whether lipid endophenotypes of the stratum corneum associate with the clinical AD subphenotypes of S. aureus colonized or barrier disrupted. In the current study, AD subjects were subphenotyped as ADStaph+ if they grew S. aureus from skin swabs obtained at lesional or non-lesional sites, while ADStaph- and all NA subjects had no growth. Skin barrier function was assessed by transepidermal water loss (TEWL) measurements of nonlesional upper extremity skin. Tape stripping adjacent to site of TEWL measurements was performed for one-step lipid extraction and lipidomics analyses using modified UP-LC methodology. Our results suggest that some lipid subtypes associate with bacterial colonization and others with physiologic evidence of skin barrier function in AD subjects. We also determined that composition of lipids was differentially altered based on the Filaggrin (FLG) mutation status. The lipid species identified in our study may play a role in barrier homeostasis and antimicrobial defense.

## P108 | Corticosteroid application prior to nickel exposure prevents contact dermatitis in sensitized individuals

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**Background:** Nickel contact dermatitis remains an important clinical problem. Patient management of nickel sensitivity relies largely on allergen avoidance, while pharmacologic treatment strategies are symptomatic but not curative.

**Objective:** We designed a double-blinded, placebo-controlled study to examine whether topical immunomodulation with calcipotriol, betamethasone diproprionate, or combined calcipotriol/betamethasone diproprionate ointments may be used to prevent recall responses in sensitized individuals and/or desensitize nickel allergic individuals.

**Method:** Participants were randomized to receive either hydrophilic petrolatum (N=5), calcipotriol (N=3), betamethasone diproprionate (N=4), or calcipotriol/betamethasone diproprionate (N=3) applied daily for 4 consecutive days. Confirmed nickel-sensitive participants were then patch tested with nickel sulfate on the extensor forearm and observed at 48 and 96 hours following treatment. Skin erythema

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was photographed and the degree of edema (1+/2+/3+) was documented at these times.

**Results:** Treatment with betamethasone diproprionate or combined betamethasone diproprionate / calcipotriol ointment resulted in strikingly visible reductions in skin inflammation in some patients on the treatment site. Ointments containing betamethasone significantly reduced patch test grades from their baseline confirmatory reaction when re-challenged at the treatment site, while vehicle or calcipotriol alone did not confer a significant effect. Irrespective of treatment, patch test reactivity remained unchanged on the contralateral forearm, suggesting a lack of antigen-specific systemic tolerance.

**Conclusions:** Repeated corticosteroid, but not calcipotriol application, prior to nickel exposure abrogated recall responses in sensitized individuals. We did not detect short-term systemic tolerance induction using either topical agents. Pre-treatment with topical glucocorticoids may be effective when used to circumvent painful hypersensitivity reactions, such as when patients anticipate exposure to a contact allergen. Further, pre-treatment with topical corticosteroids may provide additional clinical utility to prevent local hypersensitivity reactions for which T cell activation is proposed, such as those seen following subcutaneous injection of monoclonal antibodies. Since T resident memory cells are known to mediate recall responses, this work suggests topical corticosteroids may be used to modulate their activity.

### P109 | Treatment of dermatoses associated with hyperhomocysteinemia

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Published reports show daily folic acid (FA) (5-7 mg) with vitamins B6 (100 mg) and B12 (1000 mcg) improves psoriasiform patch test reactions and palmar plantar pustulosis. Psoriasis cases have been published and presented some also shown that flared on 1-2 mg daily FA, B6 and B12 yet improved when the folic acid dose was increased to 4-7 mg. Five mg FA, B6 and B12 were added to patients on 16 weeks of adalimumab, 2 of 7 patients' psoriasis worsened. Both had body mass indices under 24 and baseline vascular endothelial growth factor levels at or above 140 pg/mL [5,6].

Lower doses of FA can be pro-inflammatory through creation of monomeric endothelial NOS. High doses can be anti-inflammatory through increased amounts of anti-inflammatory conjugated eNOS, BH4 recycling, stimulation of memory T cells with folic acid receptors though IL-2, clearing inflammation though folic acid receptors on macrophages, deactivation of peroxynitrite derived radicals and inhibition of NF-kappaB.

Homocysteine (Hcy) reduces expression of VEGF-A and VEGFR-2. Reducing Hcy with 1- 2 mg daily FA may promote psoriasis by allowing VEGF effect to act unopposed.

Reducing or stopping these high FA doses may place a patient at risk for comorbid events due to the passage through pro-inflammatory FA levels. The safety of stopping this therapy requires study.

#### P110 | Initial validation of the Burden of Disease in Atopic Eczema (BODE) instrument, a quality of life measure for adult atopic dermatitis

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Atopic Dermatitis (AD) is a chronic inflammatory disorder with significant effect on quality of life (QoL). Though multiple instruments have been used to measure the impact of AD on QoL, none have been found to be adequately validated for use in clinical trials. In response, we developed the Burden of Disease in Atopic Eczema (BODE) instrument, a QoL measure that uses visual analog scales to assess AD impact on QoL. The objectives of this study were to test the structural validity, internal consistency and construct validity of the BODE. BODE was administered to 66 patients with AD at two tertiary care centers. Patients additionally completed previously validated QoL measures, Dermatology Life Quality Index (DLQI) and Skindex-16, and AD severity was evaluated with an investigator global assessment and the EASI (Eczema Area and Severity Index). Factor analysis resulted in the removal of three guestions and a revised 7-item BODE. Cronbach's alpha for the 7-item was 0.909. The 7-item BODE demonstrated excellent construct validity, with strong correlations with the DLQI (Pearson's correlation coefficient r=.738, P<.001) and Skindex-16 (r=.860, P<.001). Our initial results using the BODE instrument as a measure of QoL for AD are promising, and the BODE warrants further validation.

## P111 | The epithelial DLX3-dependent transcriptome regulates skin immune homeostasis

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Skin Inflammation is a complex process that involves extensive crosstalk between keratinocytes and leukocytes. Abnormal keratinocyteleukocyte interaction leads to chronic inflammatory skin disorders that are associated with hyper-proliferation, aberrant keratinocyte differentiation and dysregulated immune reactions. DLX3, a homeobox transcription factor, is an essential regulator of epidermal differentiation. Epidermal specific deletion of Dlx3 disrupts keratinocyte proliferationdifferentiation balance and initiates an IL-17 linked psoriatic-like skin inflammation. However the triggering signals from Dlx3-null keratinocytes responsible for psoriatic-like inflammation remain poorly understood. To identify the initial signals produced by Dlx3-null keratinocytes and to better understand the epidermal-dermal crosstalk regulating inflammatory reactions in this context, we performed -WILEY—Experimental Dermatology

an acute deletion of DIx3 in adult epidermal keratinocytes using a tamoxifen-inducible Krt14-cre/ERT system (K14CreERT;DIx3f/f). FACS analysis of DIx3f/f (Control) and K14CreERT:DIx3f/f (Mutant) skin exhibited dermal accumulations of macrophages and neutrophils within 3 day of tamoxifen-induced DIx3 ablation, with enhanced infiltration at later stages (1 week and 2 week) of DIx3 deletion. We also observed increased infiltration of IL17A secreting γδ and Vγ4 T-cells starting one week after DIx3 deletion. Expression profiling analysis by RNA sequencing of K14CreERT;DIx3f/f epidermis showed upregulation of Keratin 6, a marker of hyper-proliferation, and altered expression of epidermal differentiation complex (EDC) genes within 3 days of DLX3 abrogation. Inflammation associated genes such as IL1α, IL18, Tslp and Defb3 were also up-regulated in mutant epidermis at early time point (3 day), with subsequent production of proinflammatory cytokines IL36 and IL17. Interestingly, through gene ontology enrichment and ingenuity pathway analysis we observed significant enrichment of MAPK (Mitogen-activated protein kinases) pathway, PPAR (Peroxisome proliferator-activated receptors) signaling and canonical WNT (Wingless-type MMTV integration site) pathway associated genes. A considerable percentage of these differentially expressed genes were directly bound by DLX3 when assayed via ChIP sequencing analysis. Collectively, our results indicate that DLX3 maintains skin immune homeostasis potentially through the direct regulation of signaling pathways which are known to have a specific role in pathophysiology of inflammatory skin disorders.

### P112 | Alterations in B-cell subsets in early pediatric atopic dermatitis

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B-cells undergo maturation and class-switching in response to antigen exposure and T-cell help. Early B-cell differentiation has not been defined in early onset atopic dermatitis (AD). Differences between pediatric vs adults might be critical for elucidating early disease mechanisms. We aimed to define the frequency of B-cell subsets associated with progressive B-cell maturation and IgE class-switching. An 11-color flow cytometry antibody panel was used to determine frequencies of different B-cell subsets (IgD+CD27+ non-switched memory/NSM, IgD+CD27- Naïve, IgD-CD27+ switched memory/ SwMe, IgD-CD27- double negative/DN, CD24-CD38+ plasmablasts and CD24++CD38++ transitional), using IgD/CD27 and CD24/CD38 gating. Immunohistochemistry was performed to define skin CD20+ and CD3+ B- and T-cells. We compared peripheral blood from 27 children<5 yo and 34 adults with moderate-severe AD, as well as age-matched controls. Compared to adults, children showed T-cell predominance in skin. Total B-cell frequency was higher in controls compared to pediatric AD (38% vs 22%, P=.04) and decreased significantly with age only in controls (r=-.5, P=.03). Memory B-cells increased significantly with age (r=.64, P<.001) and correlated positively with IL-13+ Th2 cells (r=.4, P=.02) in pediatric AD. Significant levels of antigen-specific IgEs (eg milk) were observed only in AD but not control children (P<.01). Memory cells were expanded in adults (P<.01), and expression of the low-affinity IgE Fc receptor (FccRII), CD23, was higher in AD vs controls. Peripheral B-cells and T-cells are altered in early pediatric AD, but T-cells predominate in skin lesions.

### P113 | Early onset pediatric atopic dermatitis is Th2, but also Th17 polarized in skin

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Atopic dermatitis (AD) affects 20% of children and 4%-7% of adults. Paradigm-shifting discoveries about AD have been based on adult biomarkers, reflecting decades of disease activity, although 85% of cases begin by 5 years. Blood phenotyping shows only Th2 skewing in pediatric AD, but immune and barrier alterations in early lesions are unknown. This incomplete understanding of pediatric AD has limited advancement of targeted therapies for children. We aimed to characterize early pediatric AD skin phenotype, and its differences from pediatric controls and adult AD. Using immunohistochemistry and RT-PCR, we assessed biopsies from 19 AD children<5y/o with disease duration for<6 months, in comparison to adult AD and healthy controls. In lesional skin, children showed comparable or greater epidermal hyperplasia (thickness, K16) and cellular infiltration (CD3+, CD11c+, FccRI+) than adults. Similar to adults, strong activation of Th2 (IL-13, IL-31, CCL17) and Th22 (IL-22, S100As) axes, and some Th1 (IFNy, CXCL10) skewing were present in pediatric AD. Children showed significantly higher induction of Th17-related cytokines and antimicrobials (IL-17A, IL-19, CCL20, LL37, PI3/Elafin), Th9/IL-9, IL-33, and innate markers (IL-8) than adults (P<.02). IL-9 and IL-33, which are associated with allergic (peanut, dust mite) sensitization, might also have a role in the onset of pediatric AD. Despite its down-regulation in adult AD, filaggrin expression was similar in AD and healthy children. The skin phenotype of new-onset pediatric AD is substantially different from adult AD and targeting of multiple cytokine axes may be needed to effectively treat early onset atopic dermatitis in children. Although excess Th2 activation characterizes both, Th9 and Th17 are highly activated at disease initiation. Increases in IL-19 may link Th2 and Th17 activation.

## P114 | Dietary modifications in atopic dermatitis: patient reported outcomes

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Despite the lack of evidence-based recommendations, patients with Atopic Dermatitis (AD) are increasingly turning to dietary manipulations to manage their skin condition, often without consulting their medical providers. Presently, only few studies have investigated the relationship between AD and dietary modifications in adults using patient reported outcomes. We conducted a cross-sectional study that surveyed 169 AD patients from August 1, 2014 through January 31. 2015. The 61-question survey asked about dietary habits, modifications, and response to those modifications. The mean age of the participants was 43.0 (SD=16.7) and 77.5% were female. 85% of participants reported a trial of dietary exclusion. The most commonly reported foods reduced/omitted were: junk foods in 68%, dairy in 49.7%, and gluten in 49%. Improvement in skin was reported when removing the following: white flour products (37 of 69, 53.6%), gluten (37 of 72, 51.4%) nightshades (18 of 35, 51.4%), junk foods (51 of 100, 51%) and alcohol (30 of 59, 50.8%). 79.9% of participants tried adding items to their diet. The most common were vegetables in 62.2%, fish oil in 59.3%, and fruits in 57.8%. Improvement in skin was noted when adding the following: vegetables (40 of 84, 47.6%), organic foods (17 of 43, 39.5%), fish oil (28 of 80, 35%) fruits (27 of 78, 34.6%), vitamin D (23 of 65, 34.4%) and probiotics (24 of 62, 28.7%). Patients reported learning about the dietary modifications mainly by trial and error (45.6%) or the Internet (45%). Although 93.5% of patients believed it was important that physicians discuss with them the role of diet in managing skin disease, only 32.5% of patients had actually consulted their dermatologist regarding their dietary manipulations. This study highlights the significance of diet as a management tool in AD from the perspective of patients. Since dietary modifications are extremely common, the role of diet in AD and potential nutritional benefits and risks need to be properly discussed with patients.

## P115 | Histopathological characterization of trichrome vitiligo

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Trichrome vitiligo consists of an intermediate zone of hypopigmentation located between the depigmentated skin and the normal unaffected skin. It is believed that trichrome vitiligo represents a gradual centrifugal spread of hypomelanosis or a stepwise depigmentation. However, the sharp demarcation among the 3 areas, the lack of gradual changes of color, and the stability of the lesion is inconsistent with this interpretation. We sought to characterize the histopathological features of trichrome vitiligo, to discern if it represents a distinct pathogenic process compared to regular vitiligo. We performed punch biopsies of vitiligo lesions, hypopigmented (trichrome) skin, perilesional normal skin, and normal skin at least 5cm from the trichrome lesion from patients with trichrome vitiligo. We also obtained age, gender and race matched control patient skin biopsies for comparison. We performed H&E, Sox-10, Fontana-Mason, CD3, -Experimental Dermatology-WILEY

CD4, CD8 and PAS stains to study the differences in basal vacuolization, melanocyte number, epidermal and dermal lymphocytic infiltrate, and basal membrane thickness among these skin samples. The results will be presented. It is our hope that by understanding of the pathogenesis of trichrome vitiligo, we can tailor treatments specifically to this disorder, thus achieving better therapeutic results. Moreover, understanding the step-wise depigmentation of trichrome vitiligo could provide unique insights to the pathogenesis of vitiligo in general.

#### P116 | Non-steroid like phytochemical, shikonin, as a candidate drug lead for promoting inflammation-resolution in skin diseases

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Phytochemical shikonins, derived from traditionally used Chinese medicinal plant, Lithospermum erythrorhizon, have been popularly used for various skin-inflammatory diseases for more than hundreds of years. For the past 15 years, we have systematically investigated the physiological, pharmacological and mechanistic mode of actions for this phytochemical. We demonstrated that shikonins can effectively suppress the in vivo mRNA transcription via binding to the TATA box- binding protein of the TNF-α promoter in mouse skin at high dosage (2-5 µmol/L range). When used at lower concentrations (0.5-1  $\mu$ mol/L) it can down-regulate the splicing activity of TNF- $\alpha$ pre-mRNA and hence inhibit inflammatory activities in THP-1 immune cells. Transcriptomic DNA microarray analysis showed that shikonin, can differ from other test drugs, and result in specific patterns on transcriptome activity and result in a "bounce" of inhibition of various inflammatory cytokines, presumably related to the "resolution" of inflammation. More recently, we further showed that shikonin can downregulate the expression of microRNA-205 and other members of the 200 family microRNAs, and hence confer a potent stimulatory effect on epithelial-mesenchymal transition (EMT) in skin wound-healing. Interestingly, shikonin was also found to augment the expression of DAMP (damage associated molecular patterns), autophagy and necroptosis markers, and stimulate tumor cell- mediated cancer vaccine activities. These results show that shikonin can exhibit both specific suppressive or stimulative anti- and pro- inflammatory activities, depending on the treatment time period and mammalian tissue types tested. Most recently, we demonstrate that the heterogenous nuclear RNA binding protein (hnRNP A1) can serve as a key target for high affinity-binding with shikonin, involving RNA and DNA binding/interacting activity. These activities on RNA processing and metabolism may impact effectively on inflammatory responses. All of the above findings may suggest that shikonin may serve as a good inhibitor for affecting positively the inflammation resolution activities in skin, presumably useful for treating various human skin diseases.

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#### P117 | Aberrant migration of CD103+ dendritic cells as a driver of disease in Alopecia areata

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Alopecia areata is an autoimmune disease specific to hair follicles. The disease is believed to be driven by cytotoxic CD8 T cells, but the mechanisms behind their activation and the role of dendritic cell subsets in this process has not been described. The C3H/Hei mouse strain spontaneously develops AA with clinical manifestations very similar to that of human disease in about 20% of the population. The disease can be transferred to healthy mice by skin grafting or by adoptive transfer of NKG2D+ CD8 T cells. Here we demonstrate that the pathogenic NKG2D+ CD8 T cell population is heterogenous, including CD44+CD62I+CD103-CD69- central memory cells (Tcm) as well as a population of CD44+CD62l CD103+CD69+ cells with a resident memory phenotype (Trm). By adoptive transfer, the Trm- but not the Tcm population was shown to be responsible for the development of AA in recipient mice. CD103+ dendritic cells (DCs) have been shown to preferentially support Trm differentiation. Interestingly, this population was significantly increased in draining lymph nodes of AA mice as compared to healthy controls. Furthermore, CD103+ DCs of symptomatic mice displayed a distinct phenotype including elevated expression of activation markers. By injecting fluorescently labeled antigen subcutaneously and then harvesting the draining LN, DC migration from the skin can be assessed in vivo. Strikingly, there was a potent induction of CD103+ DC-migration in C3H/Hej mice, but not in WT C57/bl6 controls, whereas levels of CD11b+ DC migration was similar in the two mouse strains. These results suggest that an innate predisposition of enhanced CD103+ DC migration from the skin could be an important driver of the development of pathogenic, auto reactive CD8 Trm cells in AA.

#### P118 | Modulation of the cutaneous microbiome through Dead Sea Climatotherapy

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Introduction: Microbial inhabitants of the human skin have long been appreciated for their role in disease, and more recently for their contributions towards establishing and maintaining health. Changes in the skin microbiota have been associated with various disease states, including Atopic Dermatitis (AD), Psoriasis, Acne Vulgaris and Dandruff. Dead Sea Climatotheraphy (DSC) is an established clinical therapy that boasts up to 95% improvement in clinical status of AD patients.

Methods: We followed a cohort of 25 AD patients and 10 healthy volunteers throughout a 28 day treatment course at the Dead Sea. Relevant clinical metadata was recorded by a physician preceding and following treatment. Lesional and contralateral unaffected sites were swabbed before and after treatment. High-resolution microbial community profiling was attained by sequencing multiple regions of the 16S rRNA gene. We additionally characterized the skin fungal microbiome (mycobiome) of patients and controls by ITS amplification and sequencing.

Results: All patients included in this study exhibited significant improvement in clinical status as measured by SCORAD (P<.0001). Actinobacteria, Firmicutes, and Proteobacteria were the dominant phyla in all samples, regardless of clinical state. Significant changes in Staphylococcus spp. and Streptococcus spp. relative abundances were observed in AD patients following treatment. Patients suffering from severe AD cluster separately than healthy controls and moderate patients. This phenomenon is ameliorated following DSC. Alpha diversity metrics exhibited marked differences for both fungal and bacterial communities as a result of DSC.

Conclusions: We propose a novel mechanism for the clinical results observed in AD patients receiving DSC, based on the modulation of the skin microbiota, and suggest that exposure to certain environmental factors, including healthy dosages and wavelengths of UV, can actuate novel therapeutic approaches to harnessing the human skin microbiome and mycobiome.

#### P119 | Autoimmune blistering diseases associated with parotid mucoepidermoid carcinoma: A report of two cases

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Mucoepidermoid carcinomas (MEC) are rare salivary neoplasms most commonly affecting the parotid gland. Rare case reports have described patients with MEC affecting various glands and concomitant autoimmune disorders, including Hashimoto's thyroiditis, myasthenia gravis, systemic lupus erythematosus, and pulmonary fibrosis. although the exact relationship is unclear. We present two interesting cases of patients with autoimmune blistering diseases (AIBD) in association with MEC. The first patient initially was diagnosed with a parotid MEC; within a month of parotidectomy, the patient subsequently developed widespread oral erosions, leading to the diagnosis of pemphigus vulgaris. The second patient was initially diagnosed with bullous pemphigoid and treated with mycophenolate (initially 2gm and increased to 2.5gm daily) as well as rituximab (two doses of 1gm two weeks apart). After achieving partial remission on continued mycophenolate eighteen months after diagnosis, the patient was noted to have an enlarging nodule on her mandible, which resulted in the diagnosis of a parotid MEC. The tumor was excised and the bullous pemphigoid remained in remission. A retrospective search of patients diagnosed with MEC at Emory in the past 16 years revealed 73 parotid MECs (28% of all MECs diagnosed), and failed to reveal any other patients with both diagnoses. These are the first cases of AIBD to be associated with MEC reported in the literature. It is possible that chronic inflammatory changes in the salivary gland can result in inappropriate

interactions of the innate and adaptive immune systems resulting in triggering of autoimmunity or that an imbalance of immune regulation following systemic therapies leads to salivary gland neoplasm. Future studies directed at identifying specific infiltrating immune cells and various cytokine pathways in the salivary glands will provide further understanding of this unusual relationship.

#### P120 | Integration of transcriptome-wide clinical acne, atopic dermatitis, and psoriasis data for therapeutic target discovery and indication expansion

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Acne, atopic dermatitis, and psoriasis all are chronic inflammatory skin diseases whose moderate to severe forms together affect nearly 1 in 2 people in the US at some point in life. Although different in their signs and symptoms, all three conditions are known to share a number of molecular pathways that are dys-regulated in the disease state. In recent years, a relatively large number of clinical transcriptomics datasets have been made publically available for each of these conditions enabling the data-mining as well as comparison of lesional to non-lesional and healthy skin samples in order to indentify sets of genes whose expression significantly and consistently changes in skin disease lesions. Such clinical transcriptomics signatures can then be potentially used in a comparative manner to determine both shared and disease-specific molecular mechanisms as well as novel targets and biomarkers. We reviewed and re-analyzed 26 independent human transcriptomics datasets from the NCBI Gene Expression Omnibus that were derived from human skin biopsy samples from published clinical trials. The resulting combined dataset set comprises a total of more than 1000 human patient samples from either lesional, non-lesional, or healthy control skin. We then conducted a meta-analysis to identify a disease signature for each condition which revealed common patterns across all three diseases but also subsets of genes that were dys-regulated in a disease-specific fashion. We then explored the underlying molecular mechanisms by enrichment analysis and identified core pathways associated with disease. We conclude that seemingly very different immune mediated dermatological diseases can be compared and differentiated at the transcriptional level, providing clues to the underlying biology and pathogenesis. Our meta-analysis demonstrates that clinical transcriptomics can also provide further insight to potentially expand a drug's indication space, and to develop new biomarkers.

## P121 | Bacterial biofilm and the *S. aureus* derived protease, staphopain, are present on the skin surface of patients with atopic dermatitis

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Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by an impaired epidermal barrier, dysregulation of innate and adaptive immunity, and a high susceptibility to bacterial colonization and infection. In the present study, bacterial biofilm was visualized by electron microscopy at the surface of AD skin. Correspondingly, Staphylococcus aureus isolates from lesional skin of patients with AD, produced a substantial amount of biofilm in vitro. When analyzing the antimicrobial effects of LL-37 against S. aureus, a marked difference between the minimum inhibitory concentration (MIC) and minimal biofilm eradication concentration (MBEC) was demonstrated, indicating that S. aureus biofilms are more resistant to LL-37 mediated killing than planktonic cells. Correspondingly, confocal microscopy analysis showed that LL-37 binds preferentially to the surface of S. aureus biofilms, leading to reduced penetration into the deeper layers of the biofilm. Immuno-gold staining of S. aureus biofilm of AD skin detected the S. aureus derived protease staphopain adjacent to coccoid bacteria. In vitro, staphopain B degraded LL-37 into shorter peptide fragments. Further, LL-37 significantly inhibited S. aureus biofilm formation, but no such effects were observed for the degradation products. The data presented here provide novel information on biofilm presence in AD patients, and illustrate the complex interplay between biofilm and LL-37 in skin of AD patients, possibly leading to a disturbed host defense, which facilitates bacterial persistence.

#### P122 | Dupilumab progressively suppresses inflammation, reduces epidermal hyperplasia and increases epidermal barrier gene expression in atopic dermatitis (AD) skin

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Dupilumab, a fully human monoclonal antibody against interleukin (IL)-4 receptor- $\alpha$  that inhibits IL-4 and IL-13 signaling, improved AD signs and symptoms and suppressed inflammatory pathways in early AD trials. We evaluated the effects of dupilumab on skin biopsies from 54 moderate-to-severe AD patients randomized to dupilumab 200mg or placebo in a phase 2, double-blind trial of 16 weekly subcutaneous injections (NCT01979016). Lesional and non-lesional biopsies were

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obtained at baseline (Week [Wk]0), Wk4, and Wk16. Gene expression analyses were performed. Dupilumab improved AD disease activity and reduced pruritus compared with placebo. Common adverse events included (dupilumab vs placebo): nasopharyngitis (3/27 vs 5/27 patients), upper respiratory tract infection (URTI; 4/27 vs 4/27), viral URTI (3/27 vs 2/27), and injection-site reactions (5/27 vs 1/27). Dupilumab but not placebo shifted the lesional skin transcriptome toward the nonlesional skin phenotype. Dupilumab vs placebo shifted differentially expressed genes between lesional and non-lesional skin by 38% (Wk4) and 95% (Wk16) vs -8% and 58% with placebo (P<.001, Wk4; P<.05, Wk16). Using the Meta-Analysis Derived Atopic Dermatitis (MADAD) transcriptome, similar shifts from lesional to non-lesional skin were observed with dupilumab vs placebo (55% vs -11% at Wk4 and 111% vs 69% at Wk16). Among genes with significant decreases in mRNA expression vs placebo were those related to hyperplasia (eg K16, MKi67), T-cells and dendritic cells (eg ICOS, CD11c, CTLA4), Th2 inflammation (eg IL-13, IL-31, CCL17, CCL18, CCL26) and Th17/Th22 activity (eg IL-17A, IL-22, S100As), most notably at Wk16, with concurrent increases in expression of barrier and lipid metabolism genes (eg FLG, LOR, claudins, ELOVL3). Dupilumab vs placebo decreased the epidermal thickness of lesional skin (median percent change from baseline) at Wk4 (-23% vs -5%; P=.0013) and Wk16 (-30% vs 1%; P=.0002). The parallel progressive shift from a lesional to a more non-lesional transcriptome and improvement in skin condition with dupilumab vs placebo demonstrate IL-4/IL-13 signaling through IL-4 receptor-a is central to AD pathogenesis and barrier disruption.

#### P123 | Impact of UVA on pruritus during UVA/ B-phototherapy of inflammatory skin diseases: A randomized double-blind study

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**Background:** Phototherapy with UVB narrowband (nb) is effective in inflammatory skin disease. The addition of UVA is traditionally advocated to reduce pruritus, but lacks evidence for this recommendation. **Objectives:** The aim of this study was to assess the effect of added UVA in UVB nb phototherapy compared to UVBnb monotherapy on pruritus, disease activity, and quality of life.

Methods: In this double-blind clinical trial, 53 patients, suffering from inflammatory skin diseases with pronounced itching (Visual Analogue Scale / VAS for pruritus ≥5), were randomized into two treatment groups. One group received UVB nb (311nm) phototherapy alone and another group received a combination of UVB nb and UVA (320-400nm) phototherapy. UV therapy was performed three times per week over 16 weeks. At baseline at start of therapy, and at 4, 8, 12, and 16 weeks, pruritus (VAS and 5-D itch score), disease activity and quality of life (Dermatology Life Quality Index) were assessed.

**Results:** In both treatment groups, a reduction of pruritus, disease activity, and an improvement in quality of life was observed. However, no difference in pruritus, disease activity, and quality of life could be detected between phototherapy with UVB nb alone and the combination of UVB nb with UVA.

**Conclusions:** Phototherapy with UVB nb and the combination of UVB nb with UVA are equally effective against inflammatory skin disease and indifferent in reducing disease-associated pruritus. Given this non-inferiority for UVBnb monotherapy, the recommendation of add-ing UVA to UVBnb phototherapy of itching inflammatory skin disease should be abandoned.

## P124 | Current status of observations of malignancies in the Psoriasis Longitudinal Assessment and Registry (PSOLAR) study

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Objective: To report cumulative incidence and results of analysis of malignancies excluding non-melanoma skin cancers (NMSC) in PSOLAR. Methods: PSOLAR is a multicenter, longitudinal, observational study evaluating long-term safety and clinical outcomes for pts eligible to receive treatment for psoriasis with biologics and/or conventional systemic agents. The incidence of malignancies excluding NMSC (eg basal/squamous cell carcinomas) overall and by treatment is reported. The rules for attribution of a malignancy to a therapy define exposure based on whether pts had any exposure to a given therapy prior to the event. In cases of exposure to >1 therapy, the rule for attribution of malignancy to a treatment is ustekinumab(UST) 1st, infliximab(IFX)/golimumab(GLM) 2nd, other biologics 3rd (nearly all adalimumab(ADA) or etanercept(ETN)), or non-biologic therapy 4th, which is consistent with the pre-specified analytic plan. Analysis using Cox hazard regression was used to identify predictors of malignancy and included medication exposure defined as UST vs no biologic and biologics other than UST (primarily ADA, IFX and ETN) vs no biologic. Results: PSOLAR is fully enrolled and, as of Aug 23, 2014, has 40 388 total pt-years of follow up with 12 093 pts. Age and gender adjusted cumulative rates of malignancy (excluding NMSC) overall and across treatments were: overall 0.68 events/100 pt years of observation (PY) [95% CI: 0.60, 0.76; 274/40388PY], UST 0.51/100PY[95% CI: 0.39, 0.66; 60/12472PY], IFX/GLM (almost exclusively IFX) 0.81/100PY[95% CI: 0.58, 1.10; 41/5176PY], other biologics (almost exclusively ETN/ADA) 0.73/100PY[95% CI: 0.61, 0.88; 116/15991PY], and non-biologic therapy 0.75/100PY[95% CI: 0.56, 0.99; 57/6749PY]. Multivariate analysis, based on any time exposure, revealed that increasing age (P<.001), and previous malignancy history (P<.001) were significant predictors of malignancy. No statistically significant increased risk of malignancy with the use of any biologics was observed.

**Conclusions:** Overall cumulative rates of malignancies are comparable across treatments. Age and previous malignancy were found to be

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associated, however, no biologics or immunomodulators were found to be associated with an increased risk of malignancy.

#### P125 | Anti-inflammatory activity of a bacterial small molecule product derives from Aryl Hydrocarbon Receptor activation

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GSK2894512 (Tapinarof) is a novel, naturally derived compound that shows efficacy following topical application for patients with psoriasis and atopic dermatitis; however, the biologic target and mechanism of action was unknown at the onset of these studies. Our internal discovery efforts found that a primary activity of GSK2894512 is direct activation of the Aryl Hydrocarbon Receptor (AhR). GSK2894512 protects against imiquimod-induced skin inflammation in mice, leading to decreased II17a gene expression, a cytokine that is central to psoriasis pathobiology. We conclusively demonstrate that the efficacy of GSK2894512 is mediated through AhR because GSK2894512 does not protect against imiquimodinduced psoriasiform lesions in AhR-deficient mice. In human tissue, we show that GSK2894512 suppresses IL17A expression from CD4+ peripheral blood T cells and skin explant cultures. Thus, GSK2894512 represents a unique class of anti-inflammatory compounds with AhRdependent cytokine modulation. These studies demonstrate the therapeutic potential of a topically-delivered AhR agonist on inflammatory skin disease and provide clinical support for targeting AhR as a regulator of inflammation.

All studies were conducted in accordance with the GSK Policy on the Care. Welfare and Treatment of Laboratory Animals and were reviewed the Institutional Animal Care and Use Committee at GSK.

#### P126 | The atopic dermatitis blood signature is characterized by increases in inflammatory and cardiovascular risk markers

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Atopic dermatitis (AD) is the most common chronic relapsing inflammatory skin disease. Accumulating new evidence suggests that beyond the classic "allergic"/atopic comorbidities (ie asthma), AD emerges as a systemic disease, with increased cardiovascular risk factors (ie hypertension), and increased vascular inflammation. We thus decided to evaluate a panel of 300 inflammatory and cardiovascular risk proteins in serum of patients with moderate-to-severe AD (n=59) using an OLINK platform high-throughput proteomic assay. In order to evaluate the levels of protein up-regulation in AD serum, appropriate comparisons were made with levels in moderate-to-severe psoriasis (n=20) and healthy control (HC) subjects (n=10). Criteria of fold change (FCH)>1.3. and false discovery rate (FDR)<0.1 were used. with adjustments for age, gender, race, and body mass index (BMI). In AD, we found significant up-regulation of 43 markers as opposed to only 13 in psoriasis, as compared to healthy controls (P<.05). Only 2 markers were significantly increased (as compared to HC) in both diseases (IL-17A, CXCL9), whereas the other markers were uniquely modulated in each disease, suggesting that distinct inflammatory circulatory signatures characterize the individual diseases.

Consistent with skin expression profiles, AD, but not psoriasis, showed significant up-regulation of general inflammatory markers (IL-2), and Th2/Th22-associated products (IL-4, IL-13, CCL17, CCL22, eotaxin-1/CCL11, TSLP and S100A12). In psoriasis, the IL-17 induced marker elafin/PI3 and MCP-1/CCL2 were among uniquely up-regulated proteins in serum. Surprisingly, established cardiovascular risk markers (ie CXCL5, TNF-alpha, MMP-1, sCD40, TWEAK) were significantly upregulated only in AD but not in psoriasis.

In sum, using a proteomic approach, we detected robust AD and psoriasis blood signatures. Overall, AD showed a higher degree of dysregulation of inflammatory and cardiovascular risk markers than psoriasis, strongly supporting its systemic nature that potentially extends beyond the classic atopic/allergic associations.

#### P127 | Precision medicine in psoriasis: Machine learning and proteomics join forces to develop a blood-based test to predict response to tofacitinib or Etanercept in psoriasis patients

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New biological agents and small molecules can effectively treat psoriasis, with efficacy ranging 30%-80%. However, despite various recent FDA-approved treatments 20%-30% of psoriatic patients fail to respond to biologics. Due to its clinical heterogeneity and multiple treatment options, optimal psoriasis control is often achieved after multiple therapeutic attempts. Thus precision medicine approaches are needed in psoriasis to maximize therapeutic efficacy, improve benefit/risk ratio and reduce treatment costs. Implementing such a personalized medicine strategy requires a pre-treatment test, able to reliably predict treatment responses. To develop such a test we applied machine-learning algorithms to proteomic data obtained using -WILEY-Experimental Dermatology

a proximity extension assay. Using 259 serum samples from a phase 3 study in adults with moderate-to-severe psoriasis (OPT Compare NCT01241591). 156 protein analytes were measured before and after 4 weeks of treatment with tofacitinib (10mg BID) or Etanercept (50mg BIW). Tofacitinib is an oral JAK inhibitor being investigated for psoriasis. Response was defined by PASI75 at week 12. Data from 80% of the patients were used to train different algorithms with a bagging strategy. Predictions were obtained for the 20% remaining patients. evaluating the classifier predictive performance. The elastic-net algorithm, using only pre-treatment data, was the best performer among the methods evaluated, with average AUC values (over 500 random 20/80 data splits) of 90.8% and 91% and accuracies of 88% and 87% for tofacitinib and Etanercept, respectively, significantly above the 50% guess. Our research enables development of a tool that will reliably predict individual responses to either treatment using a pre-treatment blood test, the first such study on a large cohort of psoriasis patients.

#### P128 | Safety and efficacy of topical calcineurin inhibitors and topical steroids in atopic dermatitis in skin of color: a systematic review

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**Background:** Atopic dermatitis (AD) is a common dermatologic disease that affects individuals of many different racial and ethnic groups. The first line treatment for AD in all skin types is topical anti-inflammatory agents, including topical corticosteroids and calcineurin inhibitors. Given the increasing prevalence of AD in dark-skinned individuals, further research is needed to elucidate potential racial/ethnic variations in the efficacy of topical treatments in these populations.

**Methods:** We performed a systematic search of PubMed for all clinical trials involving "atopic dermatitis" or "atopic eczema" and "topical calcineurin inhibitor," "TCI," "tacrolimus," "pimecrolimus," "corticosteroid," or "TCS." Studies involving phototherapy, systemic treatment, or topical medications other than corticosteroids or calcineurin inhibitors were excluded. Other exclusion criteria included studies on nonatopic eczema (eg chronic hand eczema, dyshidrotic eczema), those with fewer than ten patients, manuscripts in non-English languages, and studies other than human clinical trials.

**Results:** In total, 596 articles were screened, of which 87 met our inclusion criteria. Thirty-one studies did not mention race or merely stated that several different ethnic groups were included. Only 23 studies examined greater than 20% non-white patients (Black, Asian, Hispanic, or other). Of these, only a single study on pimecrolimus cream stratified the results by ethnic group, demonstrating no difference in treatment outcome between Caucasians, Blacks, Asians, and others.

**Conclusion:** There is insufficient evidence to determine whether there are racial/ethnic variations in the efficacy and tolerability of topical anti-inflammatory medications in the treatment of AD. More research is needed to evaluate AD treatments in diverse ethnic groups.

#### P129 | Extended genome-wide association study in the United Kingdom revealing novel susceptibility loci for severe Acne vulgaris

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Acne vulgaris (acne) is an inflammatory disorder focused on the cutaneous pilo-sebaceous units. We previously reported a genome wide association study (GWAS) in 1893 cases with severe disease and 5132 controls, followed by replication in a further 2063 cases and 1970 controls. This study robustly identified three associated genomic risk loci each containing genes linked to TGF $\beta$  pathways thus suggesting a key role in acne susceptibility. To identify additional risk loci and putative disease specific biological pathways we have undertaken further genomewide genotyping of an additional 3668 cases, ascertained using identical criteria, and 6911 controls. This enlarged cohort, further strengthened by meta-analysis with the previous study, represents the largest investigation of the genetic basis of acne to date with substantial statistical power to detect acne susceptibility loci.

After appropriate quality control and imputation on the Haplotype Reference Consortium 1.1, association testing was undertaken and a meta-analysis performed combining the original and newly genotyped individuals. The three previously reported risk loci at chromosomes 1q41, 5q11.2 and 11q13.1 were confirmed. In addition, we identified several additional new associated loci achieving genome wide significance (P<5×10-8). These include loci located within the epidermal differentiation complex at chromosome 1q. These data provide strong genetic support for the role of novel barrier and innate immune pathways in acne pathogenesis as well as pointing towards potential new therapeutic targets.

## P130 | Gentian Violet: A potential treatment for cutaneous leishmaniasis

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Many types of medications have been used to treat cutaneous leishmaniasis (CL) but organic antimony compounds are mostly used to treat this endemic disease. However, those compounds are associated with cardiac toxicity and require careful monitoring. Thus, an inexpensive and safe systemic drug for Leishmania is highly needed.

Interestingly, Gentian Violet, a triphenylmethane derivative, showed a remarkable in vitro and in vivo efficacy against parasites causing CL such as Leishmania braziliensis, L. amazonensis and L. major.

In this study, we investigate the effect of three different doses of Gentian Violet (1.25, 2.5 and 5 mg/kg) given through intraperitoneal injections on L. major induced CL in BALB/c mice as to hyperalgesia, the levels of CL involved cytokines such as IFN $\gamma$ , TNF $\alpha$ , IL-4, IL-10 and IL-17 as well we the course and outcome of infection.

Our results showed that 2.5 mg/kg of Gentian Violet is the most efficient in reversing L. major-induced hyperalgesia and in reducing IL-4 and IL-10 levels. These data suggest that the Th2 response, usually associated with susceptibility to L. major infection, was impaired by Gentian Violet treatment. On the other hand, the Th1 response, which is known to lead to resistance against the disease (as assessed by the levels of IFN $\gamma$ ) as well as the levels of TNF $\alpha$  and IL-17 were not significantly affected. More importantly, 2.5 mg/kg of Gentian Violet led to healing profile as assessed by the reduced parasite burden in the infected paws of the mice. In conclusion, Gentian Violet is a potentially efficient treatment for CL by inhibiting the Th2 response which is associated with the susceptibility profile.

### P131 | Immune – Epidermal stem cell interactions in inflammatory skin disease

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Psoriasis and Atopic Dermatitis are relapsing-remitting skin diseases that result from uncontrolled immune cell activation and epidermal dysfunction. Local pools of long-lived stem cells replenish the skin epithelium throughout an organism's lifetime. Despite their central role in skin barrier maintenance, if and how epithelial stem cells are affected by and contribute to skin inflammation requires elucidation. To address this, we examined stem cell populations that maintain the skin epidermis in the context of imiguimod induced psoriatic inflammation. Lineagetracing studies revealed that Keratin14+ interfollicular epidermal stem cells (EpdSC), but not their Keratin10+ differentiated progeny, contribute to inflammation and persist long-term. Remarkably, inflammationexperienced EpdSCs displayed augmented wound-healing function mediated by their heightened proliferative and migratory capacity. This altered EpdSC behavior post-inflammation was restricted to the initial site of inflammation and did not require the presence of effector Rorc+ T cells. Epidermal stem cells underwent dramatic epigenetic and transcriptional changes during the inflammatory response; up-regulating an array of immune activating, antimicrobial, metabolic, proliferative and migratory genes. Although their transcriptional landscape was largely restored to baseline upon resolution, inflammation-experienced EpdSCs maintained chromosomal accessibility at sites associated with inflammatory response and cellular metabolism. Our findings reveal that stem cells retain an epigenetic memory of inflammation, which enables their functional adaptation to subsequent perturbations. This epigenetic rewiring may represent a learning mechanism for long-lived cells to cope with environmental stressors and contribute to the pathology of recurrent inflammatory skin diseases.

## P132 | Differences between the microbiome of lesional and non-lesional skin in atopic dermatitis

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Atopic dermatitis (AD) is a chronically relapsing inflammatory skin disease. It is still unclear whether and how changes in the microbiota - besides a reduction of the local diversity and an incease in *Staphylococcus spp.* - influence the severity of AD flairs. Here, we address the differences in the microbiome of lesional and neighboring non-lesional skin in AD patients as compared to healthy controls.

AD status was monitored and documented by a physician using SCORAD. The microbiome in skin swab samples of patients (N=11, one lesional site and an adjacent non-lesional site) and healthy controls (N=7) was sequenced using amplicon based 16S analyses of hyper variable regions V1 to V3.

The global microbiome of AD patients' skin samples is significantly different from that of healthy donors' skin samples. We confirmed that the microbiota diversity in AD lesional skin samples is significantly lower than that of AD non-lesional, and both lower than healthy donors' skin. This is mainly due to significantly higher frequency of the most abundant species, of the genus *Staphylococcus*, in AD lesional samples. However, we see no global difference between AD lesional and nonlesional skin samples. Nevertheless, there are significant differences in the frequencies of several species, in particular of the *Staphylococcus* genus, between AD lesional and nonlesional skin samples.

Our results confirm and extend the current knowledge about AD related microbiome changes. We are currently investigating whether the skin microbiome changes in AD, with respect to lower diversity and increasing Staphylococci counts, are a cause or result of the AD status. One option for future intervention or treatment could be the "self-microbiome transplantation" reducing an acute flair.