Varieties of immune cells orchestrate cutaneous immune responses. To capture such dynamic phenomena, intravital imaging is an important technique and it may provide substantial information that is not available using the conventional histological analysis. Multiphoton microscopy enables the direct, three-dimensional, and minimally invasive imaging of biological samples with high spatio-temporal resolution, and it has now become the leading method for in-vivo imaging studies. Using fluorescent dyes and transgenic reporter animals, not only skin structures but also cell- and humor-mediated cutaneous immune responses have been visualized.

In this meeting, I will introduce recent findings in cutaneous immune responses in mice and skin structures in inflammatory skin diseases using two-photon microscope.

**11β-HSD1 as homeostatic regulator of skin inflammation**

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Over the last decade, local cortisol/corticosterone production by de novo synthesis and by an activating enzyme, 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1), has been reported in various tissues including skin. We reported that 11β-HSD1 is expressed in keratinocytes and regulates inflammation and keratinocyte proliferation and wound healing \(^1\). Expression of 11β-HSD1 is decreased in benign and malignant skin tumors, including seborrheic keratosis, basal cell carcinoma, and squamous cell carcinoma \(^2\).

In this study, to investigate the function of 11β-HSD1 in keratinocytes during inflammation in vivo, we created keratinocyte-specific 11β-HSD1 knockout (K5-Hsd11b1-KO) mice and analyzed the inflammatory response in models of hapten-induced contact irritant dermatitis. K5-Hsd11b1-KO mice showed enhanced ear swelling in low-dose oxazolone-, 2,4,6-trinitro-1-chlorobenzene (TNCB)-, and 2,4-dinitrofluorobenzene-induced irritant dermatitis associated with increased inflammatory cell infiltration. Topical application of corticosterone dose-dependently suppressed TNCB-induced ear swelling and cytokine expression. Similarly in mouse keratinocytes in vitro, corticosterone dose-dependently suppressed 2, 4, 6-trinitrobenzenesulfonic acid (TNBS)-induced IL-1α and IL-1β expression. The effect of 11-dehydrocorticosterone was attenuated in TNCB-induced irritant dermatitis in K5-Hsd11b1-KO mice compared with wild-type mice. In human samples, 11β-HSD1 expression was decreased in epidermis of psoriasis vulgaris compared with healthy skin. Taking together, these data suggest that corticosterone activation by 11β-HSD1 in keratinocytes suppresses hapten-induced irritant dermatitis through suppression of expression of cytokines, such as IL-1α and IL-1β, in keratinocytes.

Uncovering novel regulators of CD8+ T-cell functions in the skin

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Cancer Council statistics show that Australia has the highest rate of skin cancer in the world (twice that of the USA and UK), and predicts 2 in 3 Australians will be diagnosed with skin cancer before the age of 70. Tumour-specific CD8+ T-cells are well-recognised for their importance in eliciting tumour-rejection, however, in many cases tumour-specific CD8+ T-cells within the tumour microenvironment are dysfunctional. The regulation of CD8+ T-cell activity in the tumour microenvironment is poorly understood. This study aimed to explore the mechanisms involved in the regulation of CD8+ T-cells in the skin as a prelude to tumour studies. We have generated a new experimental mouse model in which activated CD8β+ T-cells from donor mice were introduced into RAG1KO mice in order to assess CD8+ T-cell deregulation in the absence of conventional-regulatory T-cells (Treg). When RAG1KO mice subsequently received CD4-depleting antibody, CD8+ T-cell-mediated destruction of the ear skin occurred. However, this did not occur in mice administered control-antibody. Analysis of lymph nodes 30 days post CD8β+ T-cell transfer showed no evidence of classical CD4+FoxP3+ Treg indicating regulation is mediated by a separate, distinct cell type. Using the model, we have identified CD4+ cells, which are distinct from classical-Treg, and we are subsequently defining the mechanism by which these cells exert control of CD8+ T-cell function in the skin. Uncovering novel pathways of CD8+ T-cell regulation will shed new light onto regulatory influences of CD8+ T-cell function within tumours and yield opportunities to develop better treatment options for cancer patients.

Oral administration of a novel RORγt antagonist attenuates psoriasis-like skin lesion of two independent mouse models through neutralization of IL-17.

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Targeting IL-17 represents a highly effective strategy of psoriasis therapy, using antibodies against IL-17A and IL-17 receptor, suggesting that Th17 cells essentially contribute to development of psoriasis. Th17 differentiation depends on the key transcription factor, RORγt. Therefore, inhibition of the RORγt signalling may be a new strategy for psoriasis treatment. We demonstrate that Rorc-deficient mice did not develop IL-23-injection-dependent psoriasis-like lesions with complete abrogation of IL-17A and IL-22. Oral administration of a novel RORγt antagonist A213, whose inhibitory effect on in vitro Th17 differentiation was more potent than digoxin, resulted in attenuation of skin inflammation in two independent mouse models of psoriasis; IL-23-injection model and K5.Stat3C transgenic mouse. Increased levels of IL-17A expression were significantly attenuated in skin lesions and skin-draining lymph nodes by systemic treatment with A213 in both models. In conclusion, this result implicates new therapeutic application of RORγt antagonist for the treatment of psoriasis.
Concurrent Session 1B: Clinical Practice 1

Current Strategy for the Management of Hair Loss Diseases in Japan

Manabu Ohyama

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Hair loss diseases represented by alopecia areata (AA) and androgenetic alopecia (AGA) are commonly encountered in our clinics. In this talk, the Japanese Dermatologic Association (JDA)’s guidelines for the management of AA/AGA are introduced. The pathophysiology of alopecia areata greatly differs between acute and chronic phase. In acute phase, perifollicular cell infiltration around the bulb of anagen hair follicles is prominent, while in chronic phase, most hair follicles are in telogen phase with little cell infiltration. One of the virtues of JDA guideline for AA is that it clearly distinguishes each phase and recommends respective remedies. Briefly, anti-inflammatory treatments for acute AA and immunomodulatory therapy for chronic AA are indicated. JDA guideline also supports intravenous corticosteroid pulse therapy for diffuse and progressive AA subsets and antihistamine for AA with atopic background. Current criteria for the application of steroid pulse therapy are relatively robust. The efficacy may be further increased by more strictly defining the criteria. Accumulated evidence suggests the correlation between AA and atopic background. In fact, antihistamines are widely used for the treatment of AA cases with atopic dermatitis in Japan. For male AGA, dutasteride will be added as a treatment option to standard therapies, including oral finasteride, topical minoxidil and hair transplantation. However, only minoxidil has been recommended for female AGA, highlighting current limitation for treating this condition. (COI: None declared)

Practical aspects of bath-PUVA therapy: Gold standard phototherapy

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Photochemotherapy with psoralen and UVA (PUVA) is widely used as an effective treatment for psoriasis. Although PUVA has become less popular, however, as narrowband UVB has become more popular, bath water delivery of 8-methoxypsoralen and subsequent UVA-irradiation (bath-PUVA therapy) remains an effective alternative to systemic application and the gold standard of photo(chemo)therapy modalities. Psoralen is phototoxic, however, and thus treatment with oral and topical PUVA is limited and requires patients to be strictly protected against sun exposure after treatment. Treatment with bath-PUVA is less restrictive and patients require protection from the sun for only 2 h after psoralen exposure. The safety profile and efficacy of bath-PUVA make this therapy preferable among the three PUVA treatment methods.

Phototherapy induces apoptosis as well as antigen-specific immunosuppression. The narrowband UVB-induced depletion of pathogenically relevant T cells results from the induction of apoptosis. Narrowband UVB therapy and bath-PUVA therapy generally induce a relatively long remission period of approximately 4 to 6 months in patients with psoriasis, a relatively long remission period that might be due only partly to the induction of apoptosis. The role of regulatory T cells (Treg) should also be considered, as narrowband UVB radiation induces local and systemic immune suppression in a model of contact hypersensitivity. In our previous clinical study, we examined whether bath-PUVA affects circulating Treg in the peripheral blood of psoriatic patients; 10 healthy controls and 18 psoriasis patients who had not previously received photo(chemo)therapy were enrolled. We assessed CD4+CD25+Foxp3+ Treg in the peripheral blood of psoriasis patients before and after bath-PUVA therapy. Foxp3+Treg in peripheral blood mononuclear cells (PBMCs) increased significantly after bath-PUVA therapy in all patients. Bath-PUVA therapy also improved Psoriasis Area and Severity Index (PASI) scores and increased Foxp3+ Treg in all patients. These findings indicate that bath-PUVA restores Treg in psoriasis patients, and suggest that the clinical efficacy of bath-PUVA therapy for psoriatic patients is due to the induction of Foxp3+ Treg. The Treg functional ratio, which
was evaluated by CD4+ CD25- effector T cell proliferation with or without CD4+ CD25+ Treg, is lower in patients before phototherapy and that bath-PUVA therapy restores Treg function to almost normal levels.

In this talk, the efficacy of bath-PUVA for psoriasis, cutaneous T cell lymphoma and other refractory skin diseases will be shown and the underlying mechanisms will be also discussed.

Cutaneous lupus: Recent research

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Recent studies in cutaneous lupus in New Zealand are summarised.

A population based study showed that compared to European New Zealanders, Māori/Pacific people in New Zealand have a greater relative risk (RR) of cutaneous lupus and discoid lupus, respectively (age and sex adjusted) RR 2.47 [95% CI: 1.67, 3.67] and 5.67 [95% CI: 3.06, 11.6].

Eighty patients with cutaneous lupus were compared to a large control group. Multivariate analysis showed that vitamin D levels were not associated with cutaneous lupus with coefficient estimates of 1.69 [95% CI: -7.2, 10.6] p = 0.71. The activity and severity of cutaneous lupus as measured by the CLASI score were not related to vitamin D levels using multivariate analysis. Respectively CLASI activity and damage coefficient estimates were 0.10 [95% CI: -0.9, 1.1] p=0.84, -0.21 [95% CI: -1.3, 0.8] p=0.69.

A study of 24 patients with discoid lupus examined illness perception. This study did not show a correlation between CLASI scores and DLQI (CLASI damage: ρ = 0.27 p = 0.21; CLASI activity: ρ = 0.0.35 p = 0.09) suggesting discordance between the dermatologist’s and patients’ assessment of discoid lupus. There was a positive correlation between CLASI activity and depression (ρ = 0.41; p = 0.05).

However, by contrast, a larger study of 50 patients with discoid lupus showed a strong correlation between quality of life measured by the DLQI and increasing severity of discoid lupus as measured by the total CLASI (ρ = 0.40 p = 0.003).

Plenary 1: Clinical Research

Nicotinamide for skin cancer chemoprevention

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Nicotinamide (vitamin B3) has a range of photoprotective effects. As a precursor of nicotinamide adenine dinucleotide (NAD+), nicotinamide prevents UV-induced energy depletion and accelerates the energy-intensive process of DNA repair in human keratinocytes, melanocytes and ex vivo human skin. Nicotinamide also reduces UV immunosuppression and reduces premalignant actinic keratoses (AKs). The Phase 3 ONTRAC study (Oral Nicotinamide To Reduce Actinic Cancer) compared rates of new basal cell carcinomas (BCC) and squamous cell carcinomas (SCC) in 386 high-risk immune competent participants randomised to receive oral nicotinamide 500mg or placebo twice daily for 12 months.

Participants in the placebo arm developed an average of 2.4 new skin cancers in 12 months, compared to 1.8 in those taking nicotinamide [estimated relative rate reduction (RRR) 0.23 (95% CI: 0.04 to 0.38, p=0.03) adjusting for centre and number of skin cancers in the previous 5 years]. Similar reductions were seen in BCC and SCC, but chemopreventive efficacy was lost during the 6 months after intervention, indicating that ongoing dosing is needed. Similar magnitudes of reduction in skin cancer were seen in a
phase 2 trial in immune-suppressed renal transplant recipients, but without reaching significance in this small pilot study (n=22).

Nicotinamide has been shown to provide safe and effective chemoprevention of nonmelanoma skin cancer in a high-risk cohort. Further work is now needed to assess its effectiveness in preventing melanoma, in preventing nonmelanoma skin cancers in immune-suppressed populations, and to further clarify its mechanisms of action.

**Actinic cheilitis: Is Imiquimod 5% a viable treatment? A clinical study**

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Imiquimod possesses a multi-faceted manner of immune upregulation. This includes chemotaxis and activation of antigen presenting cells, activation of T-cells (TH1 and NK cells), interference with tumor-protective Fox-P3 lymphocytes, elaboration of various pro-inflammatory cytokines (especially TNF-alfa), and induced cellular apoptosis. Imiquimod is currently approved in the USA for the treatment of external genital warts, actinic keratosis, and superficial basal cell carcinoma; however, it has been used off-label for a variety of other disorders. Most patients tolerate imiquimod well, although exaggerated inflammation, flu-like symptoms and hypopigmentation may occur.

The drug’s efficacy in SCCIS suggests that it may be effective in the management of actinic cheilitis, an early manifestation of neoplastic transformation of the lip. This is an important entity, as SCCA of the lip is an aggressive and potentially fatal malignancy. In this open-label study, 50 Caucasian males, all aged >50, were asked to apply imiquimod 5% once daily, for a maximum of 8 weeks, to clinically recognized and histologically verified actinic cheilitis. The endpoint of therapy was heavy mucosal crusting or drop-out due to intolerance or lack of effect despite 8 weeks of use. Results: 44 of 50 (88%) patients had clinical and histologic clearing. At one year followup, all those who cleared remained clear. 4% dropped out due to pain; 8% failed either clinically or histologically. Due to frequent symptomatic adverse events, other approaches (lower concentration imiquimod or pulse therapy) should be studied.

**Concurrent Session 2A: Stem Cells and Carcinogenesis**

The novel hedgehog activator, serum response factor, is necessary for resistant BCC growth

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Basal cell carcinoma (BCC) is the most common cancer and patients with advanced disease lack effective treatment options. Hedgehog (HH) signaling is critical for BCC growth and recently approved Smoothened (Smo) inhibitors show efficacy in advanced BCC treatment. Unfortunately, tumor resistance is common, highlighting the need to better understand mechanisms of tumor evolution. To identify non-Smo mechanisms that account for resistant growth, we performed exome and RNA sequencing of resistant and sensitive tumor genomes from a novel murine model for BCC resistance and our advanced BCC patients. Here we present evidence implicating the cytoskeletal and cytokine regulated Serum Response Factor (SRF) in tumor resistance. Unbiased gene set enrichment analysis to uncover transcription factor expression signatures identified SRF as a top candidate. Additional bioinformatics and SRF chromatin immunoprecipitation analysis reveals SRF and GLI form a complex in resistant BCC cells, suggesting SRF regulates downstream HH pathway activation. Examination of human and mouse tumors reveals that the active, nuclear form of SRF is highly enriched in resistant, but not sensitive, tumors and resistant tumors that lack activating Smo mutations. In a search for known obligate co-factors that activate SRF in BCCs, we find that
myocardin related transcription factor (MRTF), but not p38 or MEK, facilitates SRF-driven HH activation and BCC growth. New pharmacological inhibitors of MRTF inhibit BCC tumor growth. Our studies link the cytoskeletal transcription factor SRF-MRTF as a novel regulator of Gli signaling and highlight SRF/MRTF as a novel therapeutic target for treating drug-resistant tumors.

**Novel strategies to reverse drug resistance in advanced SCC**

Alba Natalia Saenz Ponce¹, Yosef Landesman², Trinayan Kashyap², Alexander Guminski³, Orla Gannon¹, Nicholas Saunders¹

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Squamous cell carcinoma (SCC) of the skin or head and neck region is a major cause of cancer death in the western world. Patient with advanced SCC have few therapeutic options and SCC remains resistant to current treatments. We previously showed that SCCs are characterized by overexpression of the activating transcription factor E2F1 and the inhibitory transcription factor E2F7. E2F1 and E2F7 are mutually antagonistic in the control of transcription, proliferation, differentiation and apoptosis. In particular, we show that E2F1/7 control the sensitivity to anthracyclines in SCC cells via E2F-dependent regulation of sphingosine kinase 1 (Sphk1) and its product sphingosine-1-phosphate (S1P). In particular, we show that S1P treatment of cells induces profound anthracycline resistance. However, it was consistently difficult to reconcile the mutual overexpression of E2F1 and E2F7 with a seemingly pro-E2F1 environment within the SCC cells (i.e. pro-proliferation, differentiation-suppressive and pro-survival).

Addressing this issue we noted that E2F7 was selectively localized to the cytoplasm of SCC cells and tumors but was exclusively nuclear in normal cells and tissues. In contrast, E2F1 was almost exclusively localized to the nucleus in normal and SCC cells and tissues. The localization of E2F7 to the cytoplasm could be reversed using inhibitors of the nuclear export protein XPO1 and siRNA against XPO1. This established that E2F7 is a protein cargo for XPO1-dependent nuclear export and that this pathway is selectively activated in SCC. The inactivation of XPO1 is now possible using selinexor (KPT-330), a Selective Inhibitor of Nuclear Export (SINE) compound currently in advanced clinical trials to treat human solid and hematological malignancies. We show that treatment of SCC cells with selinexor reverses anthracycline resistance. Significantly, we show that a combination of selinexor + doxorubicin in vivo induces profound anticancer activity.

Thus, we conclude that i) E2F7 is selectively mislocalized in SCC, ii) E2F7 nuclear export is XPO1-dependent, iii) dysregulation of XPO1 causes derepression of S1P-mediated anthracycline resistance and iv) anthracycline resistance is reversed with inhibitors of XPO1 in vivo.

**Basal Cell Carcinoma development is promoted by ablation of the dermal papilla mesenchymal niche.**

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Basal Cell Carcinoma of the skin is the most common cancer in Australia and is one of the highest health expenditures. It is known epithelial-mesenchymal interactions are crucial for BCC development and therefore targeting the BCC mesenchymal niche is a promising strategy. While altered protein expression has been reported in BCC dermis, very little is known about the niche itself, including the cellular origin and
the signal mechanisms resulting in its development. Sox18 is a transcription factor expressed in a specialised region of mesenchyme known as the dermal papilla, with essential roles in maintaining hair follicle proliferation. We have shown in mice that dominant negative loss of function mutation of Sox18 (sox18DN) inhibits dermal papilla differentiation. Using this model we have combined with Sox18DN mutation with epidermal specific Patched1 mutation, a BCC model, to delete the dermal papilla from the BCC niche. We have found that dermal papilla lineage ablation promotes BCC development, linked with increased epidermal proliferation and progenitor markers such as Sox9. These results indicate Sox18 negatively regulates BCC development, indirectly, through suppression of its niche. Our studies on Sox18DN hair follicles also indicate dermal papilla loss induces epidermal cell exit from the stem cell compartment. Therefore it is likely that increased BCC development after Sox18DN mutation is due to activation of the epidermal progenitor compartment. In conclusion, genetic loss of Sox18 function promotes BCC tumour formation. Induction of dermal papilla signalling in the BCC niche may therefore represent a novel avenue for development of BCC therapeutics.

Epidermal clonal proliferative response upon UVB irradiation is bimodal.
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Epidermal cancers are the most prevalent form of malignancy, and are strongly linked to ultraviolet exposure. Although significant progress has been made in our understanding of skin cancer, its origin is to date unclear. It is not understood how photodamaged skin, actinic keratosis and SCCs can harbour the same mutations. In this project, using unique Rainbow mouse genetics technology, we aim to show how different clones of epidermal cells respond to ultraviolet radiation injury and progress. This allowed to track the fate of individual epidermal cells over long periods of chronic ultraviolet exposure.

Our findings highlight a bimodal progression of epidermal clones. Epidermal clones expanded more if attached to hair follicles (HF) (P<0.0001) compared to those not attached that remained of smaller size despite months of UV irradiation. Computer simulations supported a model where epidermal populations distant from HF contained committed epidermal progenitors that were quiescent and behaved differently in proximity of HFs to local proliferative signals.

In normal skin or after UVB exposure, the epidermis surrounding HFs was not systematically connected with the deep HF epidermal cells and labelling of Keratin15 expressing cells did not result in labelled cells in the interfollicular epidermis upon chronic UVB irradiation. Long term UVB irradiation resulted in alteration of fluorescence in the IFE distant from HF whereas HF and the surrounding IFE were preserved.

In conclusion, this dual behavior of epidermal clones suggests an accumulation of mutations due to UV irradiation distant from the epidermal adnexae with a new understanding of epidermal carcinogenesis.

Notch4 mediated MET-like transition involves crosstalk with non-canonical WNT signalling in melanoma
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Notch and WNT signalling pathways are highly conserved and play critical roles during development and tissue homeostasis. Both pathways are well established oncogenic factors in several cancer types including melanoma and a crosstalk between both has been reported to drive carcinogenesis. In melanoma the function of WNT and Notch signalling are controversial as both oncogenic and tumour suppressive functions have been reported. Here we report that overexpression of the constitutively active intracellular-domain of Notch4 (N4ICD) drives a mesenchymal-epithelial-like transition (MET) that results in a low
migratory, low invasive and low proliferative phenotype with decreased tumorigenicity in-vivo. Investigating the transcript profile of 50 EMT markers in N4ICD overexpressing cells revealed an epithelial-like gene signature with major EMT markers, including Vimentin, MMP2, Snail2 and Twist1, significantly downregulated but several epithelial markers, including E-cadherin, desmoplakin and occludin significantly upregulated. Strikingly WNT5A, considered as an EMT marker, is strongly upregulated following N4ICD induced MET. The increase in transcript levels however does not correspond to protein levels as overexpression of Notch4 suppresses Wnt5A. Similarly, overexpression of WNT5A increased transcript levels but decreases protein levels of Notch4 indicating a complex regulatory crosstalk between Notch4 and Wnt5a. Investigating publicly available microarray datasets also showed an inverse correlation of Notch4 and WNT5A for all stages of melanoma formation (normal skin, naevi, primary melanoma, and metastatic melanoma). Taken together our results show a novel function of Notch4, contributing to melanoma plasticity and uncover a previously unrecognized crosstalk between two highly conserved developmental signalling pathways in melanoma.

**Concurrent Session 2B: Procedural Dermatology**

**Advanced acne scars corrective procedures**

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Acne scars represent one of the most challenging skin alteration to treat. Each scar is different in its anatomical characteristics, as each patient is different in his/her own biological reactivity. Many classifications have been proposed to help Dermatologists to better plan their corrective strategies. Different scars require different approaches. Combination strategies, either performed during the same treatment session, or planned in multiple operations, represent the best and more successful option. Surgical procedures can be effectively combined with ablative or non-ablative Laser remodeling. Chemical peels can be added by experienced physicians. Patient selection, along with discussion of possible clinical outcomes, are crucial to avoid dissatisfaction. Patients should be aware that, in spite of all costly technologies, their skin will never go back to how it was prior to scarring. Some degree of improvement will be always achievable but its amount should be fully understood by Patient whose expectations should be kept within a realistic range. Proper anaesthesia should be implemented to make procedures more acceptable. Post-treatment wound care represents an integral part of any dermatologic procedure since any skin wounding cannot be considered stabilized until skin functional levels be fully re-established. Patients should be informed on the importance of performing their post-treatment dressing routine at home. Multiple procedures will be often required to achieve the improvements discussed during consultations. Periodically scheduled procedures will be necessary to benefit from the improvement achieved after a first series of treatment since biological modifications associated with aging contribute to make post-acne scars partially evident again.

**Thinking laterally about Melanocytic pigmentation with fractionated lasers**

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There are many lasers and IPL devices that treat pigmentation. The effects on pigmentation with lasers primarily treating other conditions can be both profound and subtle.

A knowledge of the interactions between various wave lengths and fluence on pigmentation is essential in treating the skin with energy devices.
Specifically the use of ablative fractional lasers for a reduction of congenital naevi will be illustrated together with the use of non-ablative lasers for increasing pigmentation.

Plenary 2: Clinical Research: Genodermatosses

Clinical management and experimental findings in epidermolysis bullosa

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Epidermolysis bullosa (EB) is a group of congenital dermatoses in which the patient's epidermis exhibits fragility and blister formation caused by genetic abnormalities in proteins of the basement membrane zone. Although the causative genes have been uncovered and dramatic progress has been made in elucidating the mechanisms of EB since the 1980s, these findings are only now on the verge of being translated into feasible fundamental treatments. Since EB occurs from birth, efficient daily wound lesion care and systemic supportive care are essential, not only for patients, but also for family members and medical staff. This is a big challenge for fundamental EB treatments, and three main therapeutic strategies have been emerging: gene therapies, protein therapies and cell-based therapies. Of these, cell therapies (with or without gene therapies) are the closest to clinical application. Sophisticated approaches have been investigated around the world, such as revertant mosaicism-based therapies, mesenchymal stem/stromal cell (MSC) therapies and induced pluripotent stem cell (iPSC)-based therapies. Herein we review bench-to-bed EB research, including recent approaches taken in our EB outpatient clinic and in an ongoing revertant mosaicism-based clinical trial.

Novel Specific Therapies for Epidermolysis Bullosa

Dedee Murrell¹

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Targeted therapies are being developed for generalized EB simplex (silencing the keratin 14 gene), Junctional EB (to replace the defective LAMB3 gene), and recessive dystrophic EB (to upregulate or replace the collagen VII gene). Gene therapy was successful for an adult male with generalized JEB as engineered skin from his stem cells which were grafted onto his wounds. Ex vivo gene therapy trials are underway for RDEB using a similar approach.

Cell therapy uses stem cells from the skin or bone marrow from normal unrelated donors to replace the defective gene. Bone marrow transplantation has been trialled for RDEB and JEB, with high mortality and some amelioration of their wounds, but it is not a cure. Intrallesional injections of mesenchymal stem cells have shown promise in small case series of RDEB. Allogeneic cultured fibroblasts injected intradermally were shown to increase collagen VII production. In an RCT of these fibroblasts vs their carrier solution of chronic wounds in RDEB, both the cell injections and the carrier solution resulted in impressive regeneration of new skin in generalized severe RDEB patients, an effect which continued to improve over 4 months.

Protein therapy to replace the missing collagen VII is promising for RDEB. A mouse model of RDEB found that both local and systemic intravenous injection of purified collagen VII healed the wounds.

PTC read-through drugs, which allow full length protein to be produced, are under investigation for RDEB.

In summary, there are many exciting developments for specific treatments of EB, at last.
Skin fibrosis is a common pathological condition in skin-restricted and systemic disorders, such as systemic sclerosis (SSc), localized scleroderma, keloid, chronic graft-versus-host disease, radiation dermatitis, and dermatosclerosis associated with venous ulcers. Among them, SSc is the most complicated and intractable disease with high morbidity and mortality due to extensive fibrosis of the skin and various internal organs. Therefore, the investigation for disease process underlying SSc is quite useful to elucidate the mechanism by which the pathological tissue fibrosis occurs and develop the treatment of this pathological condition. The pathogenesis of SSc is highly complicated because the three cardinal pathological features, such as immune abnormalities/inflammation, vasculopathy, and fibrosis, interact with each other, eventually resulting in the establishment of various clinical manifestations. However, we have continuously demonstrated that the deficiency of transcription factor Fli1, which is epigenetically suppressed in the bulk skin and cultivated dermal fibroblasts of SSc patients, integrates the activation of fibrosis-related gene programs in various types of cells, including fibroblasts, endothelial cells, macrophages, B cells, and epithelial cells. In this presentation, I will present a series of data regarding the impact of Fli1 deficiency on various types of cells, suggesting the potential of this molecule as a therapeutic target of fibrotic disorders.

Novel strategies for the prevention of HIV transmission

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Sexual transmission of HIV is the most common mode of infection in the global HIV epidemic. In the absence of an effective vaccine, there is an urgent need for additional strategies to prevent new HIV infections. In the initial phase of sexual transmission of HIV, virus crosses mucosal epithelium and is eventually disseminated from local sites of infection to proximally located lymphoid organs, where it establishes a permanent infection in the host. An emerging body of evidence now indicates that Langerhans cells (LCs) are initial cellular targets in the sexual transmission of HIV, and CD4- and CCR5-mediated infection of LCs plays a crucial role in spreading HIV from mucosal sites to lymphoid organs. Recently, we have shown that orally delivered CCR5 inhibitor: Maraviroc rapidly distributes to skin and functionally acts to block HIV infection of LCs. In addition, we and others have recently found that HIV susceptibility of LCs is directly and indirectly enhanced by STD pathogens, thereby leading to enhanced sexual transmission of HIV. Based on these insights, pre-exposure prophylaxis (PrEP) with oral administration of antiretroviral drugs and blockade of HIV infection-enhancing effect by STD that interfere with HIV infection of LCs might be considered as an alternate approach to decrease sexual transmission of HIV.
Regulatory B cells ameliorate the symptoms of systemic sclerosis via an antigen-specific manner: the novel therapeutic strategy for autoimmune diseases.

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Purpose: Immune cells play a critical role in systemic sclerosis (SSc). It had been thinking that B cells have only the function of antibody production. However, previous studies have revealed that B cells regulate immune responses crucially, and play important roles in SSc. Furthermore, specific B-cell subsets, regulatory B cells, can negatively regulate T-cell immune responses. Recently, we have shown that the ex vivo provision of CD40 and IL-21 receptor signals can drive regulatory B cell development and expansion. In addition, regulatory B cell maturation into functional IL-10-secreting effector cells that inhibit autoimmune disease requires IL-21 and CD40-dependent cognate interactions with T cells. However, the effects of antigen-specific regulatory B cells are remained unclear. In this study, we examined the role of antigen-specific regulatory B cells in SSc.

Methods: Bleomycin (BLM)- or topoisomerase I-induced SSc model mice were used in this study. To assess the antigen-specific regulatory B cell function, our original regulatory B cell development and expansion system and high-sensitive micro ELISA system were used, because antigen-specific B cell number was very low.

Results: In BLM-induced SSc model mice, regulatory B cells significantly ameliorated their symptoms, including tissue fibrosis and immunological abnormalities. Interestingly, topoisomerase I-specific regulatory B cells obtained from topoisomerase I-induced SSc model mice showed more inhibitory effects on these abnormalities than conventional regulatory B cells.

Conclusion: The ex vivo expansion and reinfusion of antigen-specific regulate B cells may provide a novel and effective in vivo treatment for severe autoimmune diseases that are resistant to current therapies.

High fat diet exacerbates imiquimod-induced psoriasis-like skin lesion by inducing IL-17A and inflammasomes in mice

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Psoriasis, a chronic inflammatory skin disease, turned out to be closely related with systemic metabolism. Recent studies revealed that an elevated body mass index (BMI) is a risk factor of psoriasis and assembly of NLRP3 inflammasome is produced by adipose tissue macrophages in obese subjects. We hypothesized that hyperlipidemia is involved in the pathogenesis of psoriasis and examined the effect of high fat diet (HFD) in the development of psoriasis in imiquimod (IMQ)-treated mice.

Mice fed with normal or HFD were treated with topical IMQ on their back skin. They were sacrificed at day 3 and skin and systemic conditions were evaluated. Body weight and serum lipid levels were measured routinely. Severity of skin lesions was represented by the skin score based on thickness of epidermis and degrees of erythema, induration, and scale. Skin infiltrates, cytokine levels, and activation of caspase-1 and IL-1β were examined by immunohistochemical staining, quantitative PCR, and Western blotting, respectively.
Body weight and serum level of cholesterol were significantly higher in mice fed with HFD (HFD-mice) compared with those fed with regular diet (RD-mice). HFD-mice showed the higher skin scores and increased number of neutrophils infiltrating into the lesional dermis. IL-17A mRNA expression was significantly increased in the skin of HFD-mice. Expression of IL-22, IL-23, and TNF-α mRNA was not enhanced in HFD-mice compared with RD-mice. Caspase-1 and IL-1β were activated in the skin of HFD-mice.

These results strongly suggest that hyperlipidemia is involved in the development and progression of psoriasis via IL-17A and inflammasomes.

Similarities of dermoscopic and immunological findings in alopecia areata between human and C3H/HeJ mouse

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The C3H/HeJ mouse is the most extensively studied rodent model of spontaneous alopecia areata (AA)-like disease. However, C3H/HeJ inbred mice spontaneously develop AA at a low frequency (approximately 20% by 12 months of age). To efficiently initiate AA, we therefore employed a novel method of transferring AA to male/female C3H/HeJ mice. In this method, lymph node cells were prepared from C3H/HeJ mice bearing AA lesions and were cultured with a cytokine cocktail for 7 days. Recipient mice were administered intravenously with the cultured lymph node-derived cells and developed AA lesions at a high frequency. We observed C3H/HeJ induced AA with reference to the known signs of human AA. Furthermore, we confirmed histopathological changes in each dermoscopic feature. At one week after the initiation of AA, residual hair shafts in the skin were seen transparently through the surface of the lesional skin. At 4 weeks, dermoscopy showed mild erythema, black dots, and exclamation mark/tapering hairs, especially at the margin of the hair loss lesion, indicating a high disease activity. At 12 weeks, there were many “white dots”, although some of the pores were obscure. At 1 and 4 weeks, CD4⁺ and CD8⁺NKG2D⁺ T cells were found around hair bulbs. At 12 weeks, corresponding to chronic AA lesions, although CD4⁺ T cells slightly accumulated, CD8⁺NKG2D⁺ T cells still continuously infiltrated around hair bulbs. Thus, C3H/HeJ induced AA resembled human AA in the phenotype of infiltrating T cells. In conclusion, C3H/HeJ mice with lymph node cell-induced AA can be used as the model for the human AA.

Concurrent Session 3B: Registrars’ Forum and Free Papers

Do pictures say a thousand words? Audit of email referrals in Waikato DHB, New Zealand

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Aim: standard email is an unsecure, but easily accessed form of communication between referrers and hospital services. We assessed quality of emailed referrals to dermatology registrars received between February 2016 – July 2016. Methods: all phone referrals from general practitioners and hospital doctors were documented and the number which required email advice recorded. Time to receipt of initial email and email reply from dermatology were recorded. History and clinical photographs were assessed for adequacy; the diagnosis and outcome documented. Results: 100 email referrals were received. 47/100 were from general practitioners. Median time to reply from initial email was 4.21 hours. 98/100 had at least one item of patient history on the email or had information supplied on the phone call; 40/100 satisfied all history criteria. 85/100 clinical photographs were adequate for diagnosis. The most common diagnosis was an inflammatory skin condition (47/100) with no diagnosis reached in 20/100. 87/100 were given advice,
the remainder offered a dermatology clinic appointment. Conclusion: In absence of formal teledermatology for urgent advice, email referrals are a common means of accessing dermatology services and can be useful given good history and photo quality.

Cutaneous toxicities of anti-Programmed cell Death1 antibodies combined with anti-Cytotoxic T-lymphocyte-associated protein 4 use in metastatic melanoma patients

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Introduction: Recent studies have demonstrated combined treatments with anti-programmed death-1 (anti-PD1) and ipilimumab leading to complementary activity in metastatic melanoma. To date, cutaneous toxicities of combined therapies are poorly described. We aim to describe and compare the cutaneous manifestations between the combined and anti-PD1 alone groups.

Methods: Patients with metastatic melanoma receiving ipilimumab and pembrolizumab together between January 2015 to February 2016 in Westmead Hospital, Sydney were identified and included in the study, these were compared to our previously published single agent anti-PD1 skin toxicities. Kaplan-Meier curves were used to compare the distribution of time taken to develop cutaneous toxicities between two groups.

Results: Of the 25 patients on combination, 88% developed new cutaneous lesions. The most frequent were immune-related; lichenoid (64%) and vitiligo (28%). Incidence of lichenoid reactions was exponentially early in the treatment with approximately one third of patients (SE 9.3%) developing their first lichenoid reaction within 12 days of commencing treatment. In patients treated with anti-PD1 as single agent, lichenoid reactions appeared at a constant rate with one third of patients (SE 8.3%) developing lesions within 14 months. However, the rate of incidence of vitiligo was similarly constant in both groups.

Conclusion: Adding ipilimumab to anti-PD1 treatment increases the incidence of lichenoid reactions while minimally affecting the development of vitiligo. Mechanisms leading to immune-related adverse events are affected by the different drugs used.

True incidence of Perineural Invasion in NMSC: A need for standardised reporting.

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Keratinocyte cancers (KC; that is, basal cell carcinomas (BCC) and squamous cell carcinomas (SCC)) are the most common cancers worldwide. Perineural invasion (PNI) by these malignancies is increasingly recognised as an important adverse prognostic factor. The incidence of Perineural Invasion (PNI) in BCC in the literature ranges from 0.18 to 10% and in the case of cutaneous SCC 3 to 14%. Together with an update on the PNI registry, we present the experience of the QSkin Study, the world’s largest prospective cohort study focussed on cancers of the skin (n=43,794 participants). In a subsample of QSkin participants, we reviewed the pathology reports of patients with confirmed BCC or SCC from two large service providers in Queensland. Of 8290 histology reports pertaining to BCCs, only 2542 (30.6%) mentioned PNI.
and of these only 38 (0.45%) were positive for PNI. Similarly, we reviewed 3462 reports pertaining to SCCs, of which 1610 (46.5%) reported on PNI and only 11 (0.32%) were positive for PNI. These findings highlight substantial under-reporting of an important prognostic factor for keratinocyte cancers, as the true incidence of PNI is difficult to ascertain when such a high percentage of histological reports do not describe the presence or absence of PNI.


The relationship between disease activity in discoid lupus erythematosus and quality of life

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This study examined the relationship of DLE severity assessed by the Cutaneous Lupus Disease Area and Severity Index (CLASI) with the Dermatology Quality Life Index (DQLI). Additionally, the patient’s perception of discoid lupus erythematosus (DLE) may vary from that of the clinician.

Fifty patients with DLE completed a DQLI and were concurrently examined to establish their CLASI.

There were 33 Māori/Pacific and 17 European participants. There were 42 (84%) female participants. The average (SD); age was 45.4 (±12.5), DLQI was 8.4 (±6.8) and CLASI total score (activity and damage) 20.1.

The overall CLASI (activity and damage) scores significantly correlated with the DQLI r=0.40 (p=0.003) as did the individual scores respectively, activity and damage; r=0.35 (p=0.01), r=0.38 (p=0.006).

Analysis by ethnic group demonstrated significant correlations amongst Māori/Pacific between CLASI activity/damage and DLQI respectively r=0.38 (p=0.031), r=0.34 (p=0.047), and similar but not significant correlations amongst European between CLASI activity/damage and DLQI, respectively r=0.42 (p=0.09) and r=0.37 (p=0.14). There was no difference between Māori/Pacific and European in DLQI, CLASI activity, CLASI damage (p>0.05).

Confirmatory factor analysis (CFA) suggests that CLASI damage (CFA correlation=0.61) is more likely to be associated with DLQI than CLASI activity (CFA correlation = 0.47).

Increasingly severe DLE is associated with reduced quality of life. Poor quality of life due to DLE is driven more by scarring than inflammation. Between the two ethnic groups, there is no difference in DLE severity or quality of life. Scores are concordant between patient and clinician.

Increment of circulating plasmablasts upon reactivation of varicella zoster virus in patients with herpes zoster

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Herpes zoster is a common disease caused by reactivation of varicella zoster virus (VZV). Fluctuation and role of plasmablasts upon this reactivation remains to be elucidated. In herpes zoster (HZ) patients, we noticed numerical elevation of plasmablast population by flow cytometric analysis of peripheral blood
mononuclear cells (PBMCs). PBMCs were taken from 42 patients with HZ at the initial examination, and at 1 week and 1 month after the onset of HZ. T cell/B cell subsets, serum cytokine concentration and VZV-specific antibodies were examined. Increase in plasmablast count as assessed by the expression of CD19+ CD20- CD27+ or CD19+ CD20- CD38+, serum IL-10 and IFN-γ concentration were prominent at the initial examination, followed by increment in CD8+ T cells at 1 week after the examination. CD4+ T cells and B cells gradually increased and peaked at 1 month after the onset. Notably, both IL-10 and plasmablast count were heightened in the early phase. Accordingly, we found IL-10-producing plasmablasts in some of the patients. These results suggest that plasmablasts not only participate in antibody production, but also serve as a suppressor (presumably Breg) of excess anti-viral immunity and resultant inflammation by producing IL-10 in HZ.

Characteristics and outcomes of rare subtypes of melanoma including acral lentiginous, mucosal and ocular melanoma: An 18-year retrospective analysis from a single U.S. institution

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Background: Rare melanoma subtypes include acral lentiginous melanoma (ALM), mucosal melanoma (MM) and ocular melanoma (OM). We sought to determine the characteristics and outcomes of ALM, MM, and OM at our institution.

Methods: All cases of ALM, MM, and OM from 1996 to 2014 were identified using the institution’s pathology database, and a retrospective review of medical records was performed.

Results: There were 82 ALM, 62 MM, and 62 OM over an 18-year period.

ALM occurred equally between genders, mostly in Caucasians, with a mean age of 66.1 years. Mean breslow depth was 3.04mm. Recurrence occurred in 18.3% of cases and most commonly presented as in-transit metastases. Mortality was 29.3% with mean length of follow-up 4.7 years.

MM occurred mostly in Caucasians, with a mean age of 66.7 years. At anogenital sites, it occurred more frequently in women than men. The mean breslow depth was 6.5mm. Recurrence most commonly presented as distant metastases. Mortality was 67.7% with a mean length of follow-up 2.9 years.

OM occurred equally between genders, mostly in Caucasians, with a mean age of 68.4 years. Mean tumor thickness was 1.5mm. Recurrence most commonly occurred locally. Mortality was 40.3% with a mean length of follow-up 5.2 years.

Conclusions: MM displayed the most aggressive behavior with highest recurrence rates and mortality. Molecular profiling will help further characterize these rare subtypes of melanoma.
A case series demonstrating when reflectance confocal microscopy (RCM) is useful in a clinical setting

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The aim of this presentation is to demonstrate the best use of reflectance confocal microscopy (RCM) in a dermatology clinic. A series of cases gathered from within a current research project being conducted at the Princess Alexandra Hospital (PAH) will illustrate the types of scenarios where the use of RCM provides benefits in patient comfort, patient care, clinician time and cost.

RCM is a non-invasive imaging modality which is well suited to improving diagnostic accuracy of melanocytic lesions1 and basal cell carcinomas2. Despite Queensland having a high burden of skin cancer, RCM is not used widely within clinical practice in the state. The aim of the current research project at the PAH is to provide convincing evidence that integrating RCM into clinical practice provides improved patient, clinician and system outcomes.


Inpatient Dermatology at a tertiary hospital in New Zealand

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1. Skin & Cancer Foundation Australia, North Sydney, NSW, Australia
2. Auckland City Hospital, Auckland, New Zealand

Inpatient care has an established role in the management of severe skin disease; however, globally there is a trend towards reduced inpatient dermatology beds. The aim of this study was to describe the inpatient dermatology experience at Auckland City Hospital.

This prospective observational study reviewed patient demographics, diagnoses, management and outcomes for consecutive dermatology inpatients treated over a twelve-month period. Dermatology Life Quality Index (DLQI) was recorded on admission and one week after discharge along with Investigator global assessment (IGA) of patient outcome at the time of discharge. Additional patient feedback was sought for a three-month period.

Eighty-four adults were admitted for dermatological care from September 2014 to September 2015. There were 45 males and 39 females with a mean age of 53 years. The majority of referrals were from local and regional dermatologists (71%) and general medicine (19%). The most common diagnoses were dermatitis, psoriasis and drug eruption and the average length of hospital stay was seven days.

On admission the average DLQI was 19 and was reduced by ten points at discharge (P = 0.001). Ninety-eight percent of patients were improved at time of discharge on IGA. Patient feedback was favourable in all cases surveyed.

Given our young cohort and the high impact of skin disease on life quality at the time of admission, inpatient care was valuable in achieving rapid improvement and return to productive activities of daily living. Inpatient care continues to have a role in the management of selected patients with severe dermatoses.
Australian Doctors’ Sun Protection Practices

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Introduction: The rates of melanoma and non-melanoma skin cancer (NMSC) are increasing in Australia. This is despite the implementation of various educational primary prevention strategies. Sun exposure is the most modifiable risk factor in the prevention of melanoma and NMSC. Recent research has revealed that Australia’s sun protection practices are inadequate, especially in 18 to 30-year-olds¹. Doctors’ awareness of appropriate sun protection behaviours and primary prevention strategies can be greatly beneficial to their patients. The aim of this research is to demonstrate the sun protection practices of Australian doctors and assess their awareness of the relationship between risk factors and skin cancer.

Method: An online survey was adapted from that previously used to assess sun protection practices. The survey was distributed to doctors practising in Australia via email.

Results: A total of 679 doctors have completed the survey; 29% (n=196) are JMOs or registrars, 24% are GPs and 47% are VMOs. Doctors from all states have been represented and 32% (n=218) of the respondents were from regional areas. One hundred and fifty-five doctors (23%) in the survey had previously had skin cancer. Sunscreen was listed as the most commonly used sun protection (45%; n=297). Interestingly 27% (n=173) of doctors did not examine themselves for skin cancer. Most of this group that did not self-examine was aged less than 45-years-old (61%; n=106) though 71% (n=123) reported that they tanned easily to moderately. In regards to awareness, 40% (n=258) of doctors correctly identified that melanoma incidence is increasing in all ages.


Cultural Competency Presentation

How to communicate with Japanese patients

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With the advancement in transportation technology, it has become much feasible for individuals to visit foreign countries in recent years, and population heterogeneity is becoming more diverse in many nations. While this trend is less seen in Japan, Australia and New Zealand are two nations that have experienced positive net overseas migration in recent years (OECD 2010), and these countries have relatively high proportions of the population who were born overseas.

The number of Japanese habitants in Australia/New Zealand is approximately 75,000 and 14,000 respectively, and compared to 10 years ago, the number has increased by more than 50%. Without doubt, this escalation is fortunate for both Japan and Australia/New Zealand, but cultural differences may sometimes lead to misunderstanding and unnecessary problem, and therefore, recognizing the differences between the countries is essential. In this session, we will discuss the health care system in Japan, focusing on the public health insurance that covers most citizens/residents. The role of general practitioner and medical specialists in Japan will also be discussed (there are over 6,000 dermatologists in...
Subsequently, we will present a short role-play to describe a Japanese patient. Since “patience” is a virtue appreciated by many Japanese (especially in older population), patient may sometimes withhold or insufficiently express his/her symptoms to the physician, and this may lead to false or under-diagnosis.

We hope the session will provide the dermatologists in Australia and New Zealand with insight to the cultural background of Japanese patients, and may become useful in the future practice.

**Plenary 4: Clinical/Translational Research**

**Genetic background of generalized pustular psoriasis**

**Kazumitsu Sugiura**

1. Fujita Health University, Toyoake, Aichi, Japan

Generalized pustular psoriasis (GPP) is an uncommon variant of psoriasis. About a century after von Zumbusch first described a case of GPP in 1910, the cause of the disease remains unknown. It was not considered a monogenic skin until Marrakchi et al. reported the causative gene of familial GPP in 2011. Currently, two genes, IL36RN and CARD14, which encode proteins secreted by keratinocytes (KC) or KC-localized proteins, are thought to be the causative or susceptibility genes in GPP.

GPP often occurs in patients with existing or prior psoriasis vulgaris (PV; “GPP with PV”). However, GPP without a history of PV (“GPP alone”) has also been reported. In 2013, my collaborators and I reported that the majority of GPP alone cases are caused by recessive mutations in IL36RN. In contrast, only a few cases of GPP with PV have recessive IL36RN mutations. We also reported recently that CARD14 p.Asp176His, a gain-of-function variant, is a predisposing factor for GPP with PV; this variant is not associated with GPP alone in the Japanese population. These results suggest that GPP alone is genetically different from GPP with PV. IL36RN mutations are also found in some patients with impetigo herpetiformis, acute generalized exanthematous pustulosis.

Currently, even individuals with heterozygous IL36RN mutations are considered susceptible to GPP. According to the Human Genetic Variation Browser and a previous report, 1–4% of the citizens in Japan and China have a pathogenic heterozygous IL36RN mutation. Thus, it is clinically important to analyze IL36RN mutations in patients with sterile pustulosis.

**Phenotypic and genotypic risk factors for naevi and melanoma**

**Richard Sturm**

1. University of Queensland, Brisbane, QLD, Australia

Total number of naevi and severe freckling are recognized as important risk factors for melanoma, however our understanding of the relationship between these benign melanocytic lesions is still superficial in relation to mole and melanoma morphology. A combination of approaches have been used to identify genes that influence the normal diversity seen in human pigmentation and somatic mutations that drive the formation and growth of pigmented lesions. Candidate pigmentation genes include the enzymes encoded by tyrosinase, tyrosinase-related protein-1 and dopachrome tautomerase (TYR, TYRP1 and DCT), the P-protein (OCA2) and the melanocortin-1 receptor (MC1R). Variant alleles of the MC1R gene resulting from a range of amino acid substitutions have been associated with red hair, fair skin, high degree of freckling as well as increased incidence of melanoma. Other population genetic studies have revealed specific polymorphisms within the MATP (SLC45A2) and NCKX5 (SLC24A5) protein coding regions associated with the degree of skin pigmentation. We are determining the genetic association of variant alleles with pigmentation phenotypes in a collection of adolescent twins and melanoma patients, and in
parallel through characterisation of cultures of human primary melanocytes derived from donor skin tissue selected based on pigmentation genotype. We are also conducting a study in 1200 volunteers from Queensland to determine genetic correlations with pigmentation, naevus phenotype and dermoscopic naevus subtypes in these individuals. Our studies show that variant alleles of MITF, MTAP, PLA2G6 and IRF4 genes are significantly associated with number of naevi. Notably, IRF4 was the lead gene associated with dermoscopic naevus pattern.

Sunday 28 August 2016

Morning Clinics

Direct immunofluorescence from biopsies of unaffected oral mucosa as an alternative simple and reliable diagnostic tool in ocular cicatricial pemphigoid: first prospective study on sensitivity and specificity.

Johannes S Kern, Dimitra Kiritsi, Daniel Boehringer, Kaethe Thoma, Leena Bruckner-Tuderman, Thomas Reinhard, Philipp Eberwein

Background: Ocular cicatricial pemphigoid (OCP) is a rare but often devastating chronic progressive epithelial fragility autoimmune disorder, which leads to conjunctival scarring and can result in blindness. It can affect solely the eye or present in combination with other mucosal or skin involvement. Diagnosis of OCP is challenging. Laboratory diagnostic tests such as direct immunofluorescence (DIF) from conjunctival biopsies may lead to further scarring and sensitivity varies. Serological tests have a low sensitivity. DIF from unaffected oral mucosal biopsy has been propagated as a simple alternative diagnostic tool in OCP and clinical experience is positive, but systematic data are lacking.

Methods: Consecutive series of 27 patients with OCP, normal control conjunctival and oral mucosal biopsies, as well as sera. Detailed ophthalmologic and dermatologic evaluation and follow-up, DIF from oral mucosa (23 patients) and/or conjunctiva (7 patients) and serological testing (indirect immunofluorescence, BP180-, BP230, Collagen VII-Elisa and keratinocyte extract immunoblotting) were statistically evaluated.

Results: DIF staining of oral mucosal biopsies in OCP at the dermal-epidermal junction can be subtle but distinct. Sensitivity of DIF from unaffected oral mucosa was 87% from conjunctiva 71% and serological testing 46% while specificities were 93%, 100% and 100% respectively. Predictive values and ROC curves were calculated.

Conclusions: DIF from biopsies of unaffected oral mucosa is a simple, sensitive and specific test for OCP, likely easier to obtain than conjunctival biopsies with less potential side effects; and therefore an alternative simple and reliable diagnostic tool for the laboratory diagnosis of OCP.

Skin Cancer, Queensland Style.

Jim Muir

1. Mater Hospital South Brisbane, Brisbane, QLD, Australia

Queensland has a very high rate of skin cancer. A significant portion of patients have multiple skin malignancies at presentation and continue to develop new lesions at a rapid rate. Care of these patients is divided amongst general practitioners, primary care doctors with an interest in skin malignancy and various specialty groups. A significant minority are looked after within the public hospital system. This can lead to very different management approaches and outcomes for this group of patients. Dr Muirs practice straddles private, public and on line delivery of care. By using several illustrative cases he will explore the
pit falls and successes in skin cancer management seen in the Queensland medical landscape. There will be time for audience discussion of the cases presented.

Persistent adult acne: A new paradigm is needed

Marius Rademaker

1. Waikato Hospital, Hamilton, Waikato, New Zealand

Acne vulgaris is usually considered a young person’s disease. Unfortunately acne requiring intervention continues to effect 30% of women in the 3rd decade of life, 20% of 4th decade, 10% of 5th decade and 5% of those in their 60s.

Guidelines for the management of acne are almost exclusively designed for adolescents and young adults. Unfortunately most of the algorithms used are inappropriate or ineffective for persistent adult acne. In addition there have been very few trials of specific treatment of adult persisting acne.

Whilst topical retinoids and benzyl peroxide are effective short term, compliance falls after 6-months of therapy. In female patients, hormonal therapy (e.g. spironolactone or cyproterone acetate) is effective, but should probably not be continued for more than 12-24 months. There is increasing disquiet regards the use of antibiotics in the management of acne.

We recently demonstrated the effectiveness of 8-months of isotretinoin 5mg/day, in 60 adults (25-55 years) with persistent adult acne. Lesion count fell from 11.3±8.1 to 1.3±3.1 by 32-weeks. Median remission lasted 24-months; most patients who relapsed elected to restart isotretinoin at 5-10 mg twice per week. No significant adverse effects were observed.

Low dose isotretinoin (5mg/day) is safe and effective, and should be considered first-line therapy in persistent adult acne, although it continues to be contraindicated in pregnancy. It is time to develop specific guidelines for the management of persistent adult acne.

Concurrent Session 4A: Cutaneous Biology

Further analysis of UVR-induced mutagenesis in melanoma

Graeme J. Walker

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We mainly consider susceptibility to skin cancer in terms of genes that regulate DNA repair or the production of protective pigment, although other aspects of skin biology are likely to be involved. In a screen for melanoma modifier genes using a murine model we found dramatic differences between strains in the acceleration of melanoma onset by UVR. UVR did not accelerate melanoma on some strain backgrounds. Hence there is a large genetic background effect in murine UVR-induced melanoma, and we have begun to discover gene candidates that might be involved.

To determine whether UVR-induced somatic changes may accelerate melanoma in the mice we compared the exomes of spontaneous and UVR-induced tumours from strains in which UVR was very effective. No recurrent “driver” mutations were induced by UVR, but there were changes in genomic footprints at the nucleotides adjacent C>T substitutions. We then reassessed published human melanoma exome data. C>Ts in sun-exposed melanomas overwhelmingly occurred at TpCpC (mutated C underlined). In acral and mucosal “spontaneous” melanomas they mostly occurred at GpCpG. Thus, either UVR predominantly induces only TC and CC dimers, or there are peculiarities of DNA repair with respect to specific pyrimidines adjacent the mutated cytosine. One might speculate that targeting the
specific mechanism(s) responsible for this footprint may be a way to reduce melanoma risk. Further, a predominant TpCpC as opposed to NpCpG signature in a melanoma is a good indicator of whether it, or the melanocyte from which it was derived, has incurred any sun damage at all.

Pericytes promote human skin tissue regeneration by increasing symmetric divisions

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2. Sir Peter MacCallum Dept of Oncology, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

Epithelial regeneration is attributed to the intrinsic properties of epidermal stem and progenitor cells, and extrinsic mesenchymal components. Although fibroblasts have been widely employed as the dermal equivalent (DE) in ex vivo skin regeneration for decades, dermal heterogeneity with respect to skin regeneration remains poorly understudied. We have identified a mesenchymal stem cell-like pericycle population in human dermis that can differentiate into mesenchymal lineages (i.e. osteocytes, adipocytes and chondrocytes) with the phenotype: CD45–/FAPα–/VLA-1bright/CD146+/PDGFRβ+/NG2+. Inclusion of pericytes with dermal fibroblasts (CD45–/FAPα+/VLA-1dim) in DEs promotes epithelial tissue regeneration. We demonstrate that DE populated solely with cultured neonatal pericytes (versus fibroblasts or a combination of pericytes and fibroblasts), induce epithelial reconstitution with the most homeostatic and highly polarized basal layer with increased expression of Ki67, K15 and ΔN-p63, that morphologically resembles normal human skin; with complete basement membrane and hemidesmosome assembly and enhanced synthesis and deposition of the extracellular matrix protein Laminin α5 (LAMA5) shown by us previously to promote epidermal regeneration. Notably, pericycle-reconstituted DEs display a greater proportion of symmetric cell divisions parallel to the basement membrane giving rise to two basal cells compared to fibroblast-reconstituted DEs, thereby contributing to the maintenance of the epidermal progenitor state. Current studies in our lab are aimed at defining the factors secreted by pericytes that influence symmetric versus asymmetric divisions. Our data suggest that pericytes are important microenvironmental modulators of skin regeneration with an untapped potential to improve ex vivo skin tissue regeneration for autologous transplantation.

AHR regulates FLG expression via OVOL1 in human keratinocytes: A plausible relationship between FLG and OVOL1 in atopic dermatitis.

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The loss-of-function mutation of filaggrin (FLG) is a well-confirmed genetic abnormality in atopic dermatitis (AD). Furthermore, genome-wide association studies on AD have revealed other susceptibility genes, such as OVOL1, related to epidermal differentiation. However, the relationship between FLG and OVOL1 in AD remains largely unknown. Based on our findings that aryl hydrocarbon receptor (AHR), a ligand-activated transcriptional factor, plays a crucial role in FLG expression in normal human epidermal keratinocytes (NHEKs), we hypothesized that AHR regulates the FLG expression via OVOL1. To demonstrate this, we have assessed; 1) whether the knockdown or overexpression of OVOL1 modulates the FLG expression in NHEKs; 2) whether AHR activation upregulates the OVOL1 and FLG expression in monolayer and 3D-cultured NHEKs; 3) whether the AHR-mediated OVOL1 upregulation restores IL-4-induced FLG downregulation which partly recapitulates the in vivo state of keratinocytes in AD. To activate AHR, we utilized 6-formylindolo[3,2-b]carbazole (FICZ), an endogenous AHR ligand and Glyteer, a clinically-used soybean tar. We found 1)
that OVOL1 positively regulated the FLG expression; 2) that AHR activation did upregulate the OVOL1 and FLG expression; 3) AHR ligation by FICZ and Glyteer restores the IL-4-induced FLG downregulation in an AHR- and OVOL1-dependent manner; and 4) that IL-4 blocked the cytoplasmic to nuclear translocation of OVOL1, but the AHR ligation opened this IL-4-induced blockade which resulted in the FLG upregulation. These results indicate that OVOL1 is an integral part of AHR-dependent FLG expression and that an efficient AHR-OVOL1 activator may potentiate the treatment of AD through restoring the FLG expression.

Genetic Associations of Skin Wound Healing in the Collaborative Cross

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Introduction: Wound healing is a complex multigenetic disorder with a heritable component that cannot be easily understood through single gene alteration studies. We aimed to perform a genome-wide association study with wound healing using the Collaborative Cross (CC), a mouse genetic panel of recombinant inbred lines derived from eight founders.

Methods: We introduced excisional wounds on 72 mouse strains from the CC and recorded changes in wound surface area over 14 days. Strains were categorised based on their healing speed (area under curve of wound surface over time) as a quantitative variable.

Results: The healing speed varied significantly across the 72 strains (P<0.0001). We were able to identify a peak of association reaching genome-wide significance on chromosome 6 (LOD 7.7). In this locus, the haplotype inherited from CAST/EiJ founder strain was associated with slow healing. Within this locus, 3 members of the aldose reductase (AKR) protein family harboured variants potentially affecting protein structure in CAST/EiJ founder only. AKR activity in mice with a CAST/EiJ haplotype was increased as reflected by high deposition of advanced glycation end-products known to contribute to poor healing and explaining the benefit of AKR inhibitors in diabetic wounds.

Conclusion: Our unbiased genome-wide approach has led to the identification of aldose reductase genes to significantly affect wound closure. This highlights the importance of oxidative stress and its effect on inflammation in the process of repair, allowing targeted therapy development.

MITF regulates cell adhesion and subcompartment-specific distribution of differentially cycling melanoma cells

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Differential tumour cell behaviour caused by environmental conditions, termed dynamic heterogeneity, is a prime source for drug resistance. We utilise real-time cell cycle imaging (Fucci) to study melanoma
heterogeneity. As distinct proliferative and invasive capabilities reflect variable drug sensitivities, identifying and characterizing these different responses is crucial to design effective therapies. Mouse xenograft tumours generated from cell lines with high microphthalmia-associated transcription factor (MITF) level displayed a homogeneous distribution of cycling cells throughout. In contrast, tumours generated from cell lines with low MITF levels were composed of clusters of cycling cells and clusters of G1-arrested cells. The proliferating areas were in close proximity to blood vessels, presumably characterised by oxygen/nutrient availability. Melanoma spheroids recapitulated the in vivo cycling behaviour, considering that here oxygen and nutrients are supplied by diffusion. MITF was undetectable within the hypoxic G1-arrested spheroid core, indicating hypoxia-induced MITF downregulation. Finally modulation of MITF expression impacted spheroid density, with overexpression giving rise to less compacted structures and vice versa. We conclude that MITF protects from cell cycle arrest induced by oxygen/nutrient deprivation. We hypothesise that high MITF levels prevent cell cycle arrest by two means: by reducing the cell-intrinsic propensity to arrest in response to low oxygen/nutrient and concurrently by allowing sufficient supply of oxygen/nutrients to cells. The latter may be achieved through decreased cell-cell/matrix adhesion resulting in the generation of looser tumours that allows more efficient oxygen/nutrient diffusion. These data outline how MITF-regulated dynamic heterogeneity could influence therapy efficiency, making MITF an important marker for drug design.

Epigenetic remodelling of H3K9Me3 leads to early stress induced drug tolerance in cancer

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Acquired drug resistance is not only caused by somatic mutations or drug efflux suggestive of alternative mechanisms like epigenetic changes and chromatin remodelling. We recently have identified early stress-induced multi-drug resistant cancer cells termed induced drug-tolerant cells (IDTCs) [1]. IDTCs were generated in four cancer cell lines corresponding to three different cancer types including melanoma, lung and colon cancer. We observed the transcriptional downregulation of the active histone mark H3K4me3 and repressive H3K27me3 but upregulation of the repressive mark H3K9Me3 in IDTCs compared to parental cancer cells. These data were consistent with different cancer types in response to stress (drug or nutrient starvation) which indicates that the alteration of histone marks is neither exclusive for any particular stress nor cancer type specific rather a generic response. Microarray and Q-PCR data suggests an upregulation of histone methyltransferases but decrease of histone demethylase representing the histone modifiers that direct the upregulation of H3K9Me3 and there is a dynamic expression of these modifiers in different cancer types. Probing for DNA methylation as an alternative cause of epigenetic changes of global CG and CpG island methylation showed no significant changes at the IDTC stage compared to parental populations. This suggests that distinct histone methylation patterns rather than DNA methylation is driving the transition from parental cells to IDTCs.

**Rose Bengal melanoma therapy - phototoxicity versus intrinsic cytotoxicity**

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Rose Bengal (RB) is a fluorescent compound that has been used in ophthalmology for diagnostics of corneal damage. RB is a photosensitizer and its phototoxicity is well characterized. Recently, it has been tested as an intralesional agent for the treatment of cutaneous melanoma metastases and is currently undergoing a Phase II trial. However, the mechanism of action of RB on melanoma is poorly understood.

In addition to standard assays we also used more unique approaches. We transduced melanoma cells with fluorescently labeled LC3 to visualize the accumulation of LC3II in the membrane of autophagosomes. Further, we combined the fluorescent properties of RB with live/dead stains to perform 3-colour fluorescence imaging of 3D melanoma spheroids.

RB had a dose-dependent cytotoxic effect on melanoma cells but not fibroblasts in the absence of light or upon exposure to red light (633 nm). In contrast, exposure to UV or green light (561 nm) caused profound phototoxicity. In 3D melanoma spheroids, RB had a time- and dose-dependent effect on melanoma cell death. In addition, RB exerted its toxicity through necrosis without perturbation of the cell cycle and the effects observed in the dark were independent of the phototoxic generation of ROS. Finally, we showed that RB induced autophagy in melanoma cells indicating a possible mechanism of action.

In contrast, to RB’s phototoxicity its intrinsic cytotoxicity has a wider therapeutic window. Here we showed that an interplay of necrosis and autophagy is one possible mechanism of action for RB.

**Cutaneous Manifestations of Helicobacter cinaedi bacteremia**

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*Helicobacter cinaedi* (*H. cinaedi*) is an emerging Gram-negative spiral bacillus that was first reported in 1984. It has been implicated as a cause of gastroenteritis and bacteremia in immunocompromised individuals. *H. cinaedi*-associated bacteremia sometimes is accompanied by skin lesions; however, the cutaneous manifestations of this pathogen are not widely known. We have conducted a retrospective review of 76 cases of *H. cinaedi* bacteremia patients at a single institution to clarify the characteristic cutaneous features. Our review of the large number of patients revealed that approximately 30% of the *H. cinaedi* bacteremia cases had sudden onset of erythema accompanied by high fever. Importantly, the most common cutaneous symptom of *H. cinaedi* bacteremia was mild cellulitis, appearing as multiple painful infiltrated erythemas on the extremities. As *H. cinaedi* is not always detectable in routine blood culture techniques, evaluation of these characteristic cutaneous manifestations seems important in diagnosis. *H. cinaedi* infection should be added to the diagnostic list of unspecified fever with painful infiltrated erythemas.
Testing of viable clonal human skin cell cultures as an approach to validating microsampling of naevi

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Skin biopsies are a valuable technique in the diagnosis of cutaneous cancers. We were interested to test the minimal size or equivalent volume by dilution of proteolytically disassociated tissue required to allow the isolation and propagation of cutaneous cells, for freezing, storage and biochemical analysis. For dispase II/collagenase processed skin tissue, the cut-off size for culture of cells was 0.1 mm³ for fibroblasts (FB), with only 1 of 48 cultures growing to confluence in a T75 flask. Melanoblast (MB) cells' smallest size for propagation was 0.5 mm³. Tissue specific antigen expression of these cultures was tested by western blot of total protein extracts. There was no DCT expression in FB cells or upon differentiation of MB cells to melanocyte (MC) cells. Smooth muscle actin (SMA) protein expression was high in FB cells, but was absent from MB, MC and malignant melanoma (MM) cell lines. E-cadherin was strongly detected in MB and MM, but was not detected in FB, and decreased in MC. Using this technique with a CDK4-R24C expressing lentivirus to overcome senescence, we have isolated and propagated naevocytes and peri-lesional (PL) melanocytes from patients with known pigment genotypes. We have compared mutational status from these cells to that of whole tissue and saliva samples from the same patients. BRAF-V600E was detected at a much higher level in the naevus cells than in the lesional (L) tissue by Sanger and Whole Exome Sequencing. Protein expression assayed for cultured L, PL, and primary MB cells, indicating higher expression of p15, p16, BRN2 in naevocytes.

Concurrent Session 4B: Clinical Research

Thinking Outside the Box! Totally Radical Innovative Therapeutics: Based on the Basics

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Understanding the basic and fundamental properties of various agents and/or devices can lead to innovative (and typically off-label) therapeutic use of the latter. A well-known example is the adaptation of antimalarial drugs in the treatment of autoimmune disorders due to the drugs' ability to interfere with antigen presentation. This talk will highlight some interesting, rather radical, treatment suggestions which are mostly based upon known properties of the therapeutic agent. The degree of evidence is NOT overwhelming. But such recommendations offer the clinician alternative, attractive (simple, safe) treatment modalities to consider when traditional therapies fail or are not well tolerated.

Innovative treatments discussed include: oral zinc (ZnSO4 10mg/kg/day) for aphthosis, warts and molluscum, N-acetyl-cysteine (up to 4800 mg/day) for addictive obsessive-compulsive disorders (such as trichotillomania and excoriation disorder), high dose Vitamin D for chronic spontaneous urticarial, fexofenadine for alopecia areata, minocycline and infliximab for sarcoidosis, nitroglycerin patch for chondrodermatitis, liquid nitrogen cryoanalgesia for post-herpetic neuralgia, topical zinc sulfate 4% for herpes progenitalis, smoking cessation for chronic MRSA infections, low dose (1-6mg/day) chlorambucil and weekly use of low-fluence (300-600 mJ/cm2) excimer laser for granuloma annulare, and pre-event intra-oral ice installation to prevent stress related flushing.
Identification of causative genes and development of pathogenesis-based therapies in genodermatoses

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Recent progress of next generation sequencing enabled us to identify new causative genes for rare genodermatoses. In this talk, I will introduce our current strategies for (1) the genetic diagnosis of genodermatoses in the clinic, (2) the hunting for new causative genes or new disease entities for genodermatoses, and (3) the development of pathogenesis-based therapies. For the genetic diagnosis, we perform a next-generation sequencing using custom targeted exome sequencing panels containing over 500 genodermatosis-related genes. We evaluate the results of the initial screening with clinical phenotypes and family histories and select patients/families for further analyses using whole exome sequencing and SNP arrays. Recent progresses in the dermatologic field demonstrate it fruitful to analyze the pathogenesis of rare genodermatoses not only for the development new therapeutic strategies but also for understanding the pathogenesis of common skin diseases.
**Poster Presentations**

**Preventing pathergy of pyoderma gangrenosum during skin cancer surgery: utilisation of intra operative intra lesion all corticosteroids**

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**Nine primary melanomas in a young female patient**

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This case study presents the history of a 34 year old female with nine primary melanomas since the age of eighteen. Melanoma remains a significant cancer burden in Australia. The Queensland Cancer Registry reports that melanoma is the most common form of cancer diagnosed in young Queenslanders and approximately 6% of melanoma patients will go on to develop a second primary melanoma.¹ The risk of developing a second primary melanoma is estimated at 4-9%, however the risk for the development of subsequent melanomas is 1% per year and remains unchanged.² The patient in this case had her first melanoma diagnosed at the age of 18, then during her first pregnancy, 16 years later, she developed her second melanoma. Over the ensuing 18 months the patient went on to present with a further 7 histologically confirmed cutaneous melanomas. The patient’s risk factors include; dysplastic naevus syndrome, type one skin, living in tropical Australia since birth and we are awaiting genetic studies. The relationship of melanoma development to her pregnancy will be discussed.


**Identification of unique molecular signatures of differentially cycling tumour cell subpopulations in a 3D melanoma model**

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Intra-tumoral dynamic heterogeneity is a leading cause of drug-resistance development in melanoma. The microenvironment plays a major role in giving rise to phenotypically different cancer cell subpopulations within a particular tumour. Therefore, understanding the molecular signatures of dynamic heterogeneity is crucial to design effective therapies. We generated 3D spheroids from fluorescent ubiquitination-based cell cycle indicator (FUCCI)-transduced melanoma cell lines. The spheroids autonomously segregated into two differentially cycling subpopulations: G1-arrested cells in the centre...
and cycling cells in the periphery. Confocal microscopy of sectioned spheroids revealed that Microphthalmia-associated transcription factor (MITF)-expression co-localises with the cycling population. To elucidate the molecular mechanism behind this phenomenon, we isolated cells from each subpopulation by Hoechst dye diffusion and FACS, validated by the MITF expression pattern. Subsequent analysis of their respective RNA (real-time PCR and RNAseq) revealed 1,592 differentially transcribed genes between these two subpopulations. The melanocyte-specific isoform MITF-M and several upstream and downstream pathway components of MITF, DNA repair and cell cycle promoting genes were significantly down regulated in the central G1-arrested population compared to the peripheral proliferating cells. Hypoxia-inducible and tumour-suppressor genes showed the opposite pattern. Pathway analysis of the RNAseq data showed differential transcription of many PI3K-AKT pathway components, indicating that this pathway is active in the proliferating periphery but shut down in the G1-arrested core. The PI3K-AKT pathway is important for survival and indirectly mediates MITF expression. Our ongoing studies aim to decipher the regulatory mechanism behind this link to gain a deeper understanding of melanoma’s dynamic heterogeneity and eventually drug resistance.

A Case Study of Granuloma Fissuratum

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Background: Granuloma fissuratum (also known as acanthoma fissuratum or spectacle-frame acanthoma) is a skin-coloured papule with a central groove that arises in areas of focal pressure and friction associated with ill-fitted prosthesis. Lesions will heal within weeks to months after discontinuing or proper adjustment of the prosthesis.

Case Report: We report a case of Granuloma Fissuratum on the inner canthus of an 84-year old lady that presented to the Royal Brisbane & Woman’s Hospital dermatology outpatient clinic. A skin-coloured papule had developed over a 6-month period following daily use of new spectacles. It was noted to have a central groove that correlated with the rim of her spectacles. Biopsy was performed and confirmed a benign, reactive process. Histology showed diffuse epidermal hyperplasia with no evidence of atypia. She was referred to an optometrist to have her spectacles adjusted and the lesion regressed over the following months.

Conclusions: Granuloma fissuratum is most common over the inner canthus or retroauricular areas from poorly fitting spectacles, but can also be seen in the mouth from dentures. The lesion will resolve spontaneously within weeks of correction of the ill-fitting prosthesis. The most important aspect of managing these patients is differentiating the lesion from a neoplastic process such as basal cell carcinoma and avoiding unnecessary surgical treatment. An aspect in clinical examination of this lesion that does not appear to be documented in the literature is the use of dermoscopy in these lesions and further research is needed in this area.
Clinical and immunological features of recurrent herpes zoster

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Background: Recurrent herpes zoster (HZ) is thought to be rare, but there have been few large-scale studies of recurrent HZ.

Objective: We conducted a large-scale prospective cohort study to characterize recurrent HZ.

Methods: We examined 12,522 participants aged 50 years or older in Shozu County and were followed for 3 years. We compared the incidence of HZ and post-herpetic neuralgia (PHN), severity of skin lesions and acute pain, cell-mediated immunity (CMI), and VZV (varicella-zoster virus) -specific antibody titer between primary and recurrent HZ.

Results: A total of 401 participants developed HZ, including 341 primary and 60 recurrent HZ patients. Skin lesions and acute pain were significantly milder and the incidence of PHN was lower in patients aged 50 to 79 years with recurrent HZ than in those with primary HZ. VZV skin test induced a stronger reaction in patients aged 50 to 79 years with recurrent HZ than in those with primary HZ.

Limitations: Information on previous HZ episodes was self-reported by participants, so it could not be confirmed that they had actually had a history of HZ.

Conclusion: Recurrent HZ was associated with milder clinical symptoms than primary HZ, probably due to stronger VZV-specific CMI in the patients with recurrence.
3D Bio-printed dermal scaffolds: Cell-scaffold interaction

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**Background:** The 3D bio-printing of dermal scaffolds combines traditional tissue engineering and cell culture principles, with more recently developed innovations in bio-fabrication, in an effort to realize the rapid fabrication of Human Skin Equivalents (HSE), for clinical and research applications. Compared with manual deposition, bio-printing offers superior control of geometric detail and the ability to integrate multiple synthetic or natural materials. Tunable biopolymers allow manipulation of the biomechanical properties and degradation profile of the scaffold. Our study applies a newly devised technique in keratinocyte culture, to 3D bio-printed dermal scaffolds, to evaluate cell-scaffold interaction for different polymers.

**Objectives:** The objectives of our study were:

1. To develop an epidermal model using 3D keratinocyte culture techniques,
2. To compare epidermal stratification and induction of basement membrane formation, for different polymers, and
3. To evaluate the putative role of Type IV collagen for the induction of basement membrane formation, in the context of 3D epidermal models.

**Methods:** Dermal scaffolds were printed using a micro-extrusion process with polymers that were optimized for their printability and tunable qualities. The scaffolds were seeded with primary human keratinocytes, and cultured using a feeder-free, serum-free protocol. Samples were extracted and analysed, at weekly intervals, to determine the longitudinal pattern of differentiation.

**Results:** Preliminary histological and immunohistochemistry analyses will be presented, comparing markers of epidermal differentiation and induction of basement membrane formation, particularly key molecules associated with hemidesmosomes, desmosomes and focal adhesion complexes.

**Conclusion:** These results will guide future work developing an ideal full thickness skin substitute.

Congenital Hemangioma presenting with acute hemorrhage- A case report and review of the literature.

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Hemangiomas are true neoplasms of endothelial cells and should be differentiated from vascular malformations which are localized defects of vascular morphogenesis¹.

Hemangiomas are the most common benign soft tissue tumor of infancy and childhood, occurring in 12% of all infants and are grouped into Infantile Hemangiomas (IHs) and Congenital Hemangiomas (CHs). Congenital Hemangiomas are usually present as solitary lesions at birth and are rare, with a combined incidence of less than 3% of all IHs.² Complications include ulceration (with or without bleeding) thrombocytopenia and heart failure. A life-threatening hemorrhage is a very rare complication of cutaneous hemangioma in the absence of thrombocytopenia and coagulopathy, except during surgery. We report a case of acute hemorrhage from a congenital hemangioma.
Methods: Case Report and Literature Review Inpatient Review of a young infant in USA. We searched PubMed with the words 'hemorrhage/bleeding + hemangioma'. All the relevant articles were reviewed.

Objectives: To report on a healthy young infant who had acute bleeding from a congenital hemangioma.

Conclusion: Congenital Hemangiomas are rare and many questions about their pathogenesis and management remain unanswered. It must be borne in mind that these lesions behave differently from the more common infantile hemangiomas. Although most bleeds from CH can be controlled with pressure, one must be conscious of the fact that life threatening hemorrhage can occur. Further awareness and familiarity with these uncommon congenital lesions will facilitate accurate diagnosis and management.


Infectious eczematoid dermatitis secondary to spider bite: Tele-derm National case report

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We present a case of a 68 year old female who presented with inflammation, swelling and desquamation of the right, 5th, distal phalanx, 2 weeks following a spider bite at an urban GP practice. Associated vesicles and clear exudate was also visible. With the assistance of Tele-Derm National, the diagnosis of infectious eczematoid dermatitis (IED) and a management plan was implemented by the primary doctor. IED is an inflammatory, eczematous, autosensitisation response to a primary, exudative site of infection [1]. The main differentials include other more common eczematous process such as allergic contact dermatitis, atopic dermatitis, and secondarily infected dermatitis. This distinction is important as the treatment for IED focusses around antibacterial agents [1, 2]. Many skin diseases can present as a diagnostic dilemma for doctors in rural and remote communities where access to specialist assistance is limited for both doctor and patient. Tele-Derm National is a teledermatology service developed in 2003 by The Australian College of Rural and Remote Medicine (ACRRM). It was designed for doctors in rural and remote areas to have fast, easy access to specialist opinion from dermatologists [3]. Tele-Derm National can be made available for any doctor however, so it may also be useful for those working in hospitals or urban GP practices. This is an example of how Tele-Derm National can be used to benefit clinical practice in Australian rural and remote communities.

Skin Cancer Management in Heart and Lung Transplant Recipients

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Background: Skin cancers are the commonest malignant condition in solid organ transplant recipients\(^1\). The rate of skin cancer in the lung and heart-lung transplant patients in Western Australia is more than 12 times higher than international populations.\(^2\,^3\)

Aim: We assessed patient awareness and frequency of skin cancer prevention management in lung, heart, and heart-lung transplant recipients in Western Australia to determine if lack of awareness and/or prevention management were contributing factors to the increased skin cancer risk identified in this population.

Methods: A de-identified questionnaire was used for data collection. All heart, heart-lung and lung transplant patients aged > 18 years, who were >1 year post-transplant and followed up by the WA state transplant units were included.

Results: We received 94 complete responses out of 163 sent (58% response rate). Of these, 41%/53%/6% were lung/heart/heart-lung transplant recipients respectively. 98% reported that they were aware of the increased risk of skin cancer. Only 69% actively took steps in daily life to prevent skin cancer. 84% reported that they have had a full skin check by a medical practitioner within the last 12 months and of the 84% reviewed, 37% were by GPs, 49% by hospital dermatologists, 31% by private dermatologists and 5% by plastic surgeons respectively.

Conclusion: The majority of transplant recipients were aware of the increased risk of skin cancer. However, individual adherence to preventative measures was suboptimal and may be a factor in the high rates of skin cancer in WA. Further work to improve adherence is needed.


MITF modifies invasion and characteristics of the epithelial to mesenchymal transition in melanoma

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Melanoma drug resistance may be due, in part, to dynamic heterogeneity. Cancer cells within a tumour exhibit various phenotypes in response to environmental stress. This results in populations with different proliferative and invasive capabilities and drug sensitivities. Understanding the molecular signature of dynamic heterogeneity is crucial to design effective therapies.

Using the fluorescence ubiquitination cell cycle indicator (FUCCI), which visually delineates the cell cycle phases, we have recently shown that in 3D melanoma spheroids the centre cells arrested in G1 phase, while the peripheral cells continued to proliferate. In melanomas expressing microphthalmia-associated transcription factor (MITF), MITF was high in the proliferating periphery but low to undetectable in the G1-arrested centre. Not only do spheroids express MITF around the perimeter, but also markers of the Epithelial to Mesenchymal Transition (EMT), such as Vimentin, Slug and N-Cadherin. Vimentin was confined to the very edge of the spheroids and the cells invading into the matrix, supporting the “classic” idea of EMT. However, Slug and N-Cadherin were upregulated in the proliferative zone. Upon MITF overexpression in...
MITF-low melanoma cells, MITF and Slug became expressed homogenously, and these spheroids in fact showed reduced invasion into collagen. We are currently exploring what other means of cancer cell migration could trump an enhanced EMT phenotype and slow invasion in our model.

These data outline how dynamic heterogeneity, including proliferative and invasive potential, is tightly intertwined with MITF expression, making it an important marker for therapy design.

Lichen nitidus: a case report of a rare condition, displaying atypical dermoscopic features.

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Introduction: Lichen nitidus is an uncommon skin condition that has been sparsely reported in the literature. We present a case of lichen nitidus with atypical clinical and dermoscopic features, displaying distinct appearances not previously described in the literature.

Method: We examine a single case report of an otherwise well 32 year-old Caucasian male with an unusual looking, asymptomatic rash that was chronic in nature on the distal dorsal fingers, dorsum of the left hand and bilateral medial forearms. Dermoscopy was non-specific, showing multiple scattered pink clods, with a surrounding pale rim. Skin biopsy was performed to obtain diagnosis.

Results: Dermoscopy of lesions on the fingers, hands and forearms were identical to one another. In addition to this, the features were distinctly different from that seen in palmoplantar lichen nitidus described in the current literature. Histology findings supported that of lichen nitidus.

Conclusion: Lichen nitidus is poorly described, with variance in its clinical presentation and dermoscopic features. We present a pattern of dermoscopy not previously characterised in the literature. The distinguishing features of this case are the monomorphic appearances of the lesions, independent of anatomic site. This may, in the future, aide the identification of non-palmoplantar lichen nitidus by other clinicians.


Medical perceptions of dermatology teaching: A focus group study

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Dermatology teaching at tertiary institutions is considered either inconsistent or inadequate despite its importance in primary care practices. The restrictions placed on dermatology teaching are most likely secondary to multiple factors. These include the massive increase in medical student numbers and the perception that dermatology is of lesser importance. This occurs on a background of an already overcrowded syllabus that prioritises other subjects.

Students’ motivation and ability to learn of a subject is strongly affected by the perceptions towards it (2). For example, students find neurology, histology and anatomy difficult because of the unique nature and amount to recall required. There is evidence that emerging innovative educational strategies are particularly useful for these subjects which are considered unique or challenging. To address the
incomplete teaching of dermatology, and to redesign its pedagogical platform, it is firstly important to consider the perceptions towards it.

The aim of our research is to conduct focus groups with medical professionals who are involved with dermatological presentations, namely GPs and paediatricians. We hope to ascertain their opinions on the relevance of dermatology, how they have supplemented their dermatology knowledge since university and what they prioritise in a dermatology curriculum. We also aim to hold focus groups with medical students to identify their perceptions of dermatology and what they consider important features in an innovative curriculum.


Origin of vascular progenitors driving melanoma angiogenesis in vivo

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The development of new blood and/or lymphatic vessels is a pre-requisite for Melanoma growth and spread through metastasis. With the view to better identify and characterize the source of de novo endothelium in cancer, we utilised flow cytometry and immunofluorescence to characterise endothelial populations in B16 melanoma. We identify endothelial cells derived from resident vascular beds and not from hematopoietic lineages. Among Lin-CD34+ cells, expression levels of VEGFR2 and CD31 defined three distinct endothelial sub-populations. Furthermore, using lineage tracing of VE-cadherin expressing endothelial cells (Cdh5-Cre/ER RosaYFP), we demonstrate a maturation sequence from progenitor (P) via transit amplifying (TA) to fully differentiated (D) cells in B16 melanoma as well as in skin wounds. Sox18, a transcription factor essential in vascular development was then considered as a functional marker of the progenitor population. Sox18 expression is lost in the adult, with reexpression reported in endothelial cells only in pathogenic situations of angiogenesis. We utilized Sox18CreER/Rosa-YFP reporter mice and showed that progenitors activate Sox18. Fate tracking over multiple tumour time-points revealed that by day 5 after melanoma cell injection, only progenitors have been recruited to the tumour; by day 10, there is a sequential progression to fully differentiated cells. Immunohistochemistry for vascular markers on these tumours demonstrate that progenitor cells originate from venous vascular beds and can give rise to de novo arterial, venous and lymphatic structures in the tumour. This highlights the usefulness of strategies targeting vascular progenitors in cancer.

Complete resolution of distant metastasis of cutaneous squamous cell carcinoma following definitive radiotherapy.

Emily Forward, Luiz P Barros de Moraes, Ivan Burchett, Stephanie Nichols, Gerald Fogarty

Recurrence of cutaneous squamous cell carcinoma (cSCC) in surgical sites is seldom reported. Patients with this condition usually have surgical excision of the metastasis. The presented case is a 77-year-old immunocompetent male who developed a four centimetres (cm) solitary symptomatic metastatic cutaneous squamous cell carcinoma (cSCC) in a split thickness skin graft (STSG) donor site on the right thigh from a scalp primary 3 months after graft harvesting. There was a complete response following
definitive radiotherapy until death from further disease six months later. Radiotherapy, can be an alternative to surgery on cSCC metastasis.

Figure 1: Fine needle aspiration biopsy of right thigh metastasis. A group of malignant keratinising squamous cells characterised by dense, intensely eosinophilic and orangeophilic cytoplasm in the Pap stained smear. Nuclei are typically large, centrally placed, angular and irregular, with coarsely granular, sometimes ink-black chromatin. The background is necrotic. (Papanicolaou stain x40)

Figure 2: Planning CT-scan for radiotherapy planning with dosimetry: The red shaded circle is the gross tumour volume and encompasses the right thigh mass. It is entirely covered by an isodose of at least 40 Gy from an opposed pair of radiation fields.

An unusual case of mixed drug eruption to pirfenidone prescribed for idiopathic pulmonary fibrosis

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We present an unusual polymorphic drug reaction in a 70-year old man taking pirfenidone, a novel antifibrotic, anti-inflammatory drug for idiopathic pulmonary fibrosis.¹ The pruritic eruption consisted of
mixed clinical morphologies: 1) eczematous eruption on the face, dorsal hands and legs with photo-exacerbation and discoid plaques on the lower legs; 2) livedo on the torso and limbs; 3) fixed urticarial plaques on the upper back. He has discontinued pirfenidone three times because of the severity of the pruritus and rash. Each occasion there has been progressive worsening over weeks of exposure, with spontaneous improvement and resolution after drug cessation, but recurrence after recommencement. Histology showed features of subacute psoriasiform dermatitis.

Pirfenidone is one of only two Therapeutic Goods Administration (TGA)-approved therapies in Australia for idiopathic pulmonary fibrosis – a progressive, irreversible, fatal disease. It can slow disease progression and potentially improve mortality.\(^2\) Trials identified rash and photosensitivity in 32% and 12% of pirfenidone-treated patients versus 12% and 2% of placebo-treated patients respectively\(^1\). Rashes led to discontinuation of pirfenidone in only 5 of 345 patients (1.4%)\(^1\).

This case illustrates how cutaneous drug reactions can present with multiple, mixed clinical morphologies. Dermatologists should be aware of reactions to this emerging drug.


Expression of different immune system regulators in stress induced drug tolerant melanoma cells

**Deepesh Gupta, Dinoop Ravindran Menon, Heinz Hammerlindl, Miranda Coleman, James Wells, Helmut Schaider**

Treatment of mutant BRAF melanoma patients with combined BRAF/MEK inhibitors inevitably leads to disease recurrence in most of the patients due to permanent drug resistance. The transition of parental cells to drug resistant cells is accompanied by changes in immune regulators expressed by melanoma cells. We recently have identified early stress induced drug tolerant cells (IDTC)\(^1\). IDTCs precede permanent resistant cells and for the former, expression of immune regulators is unknown. To this purpose, IDTC have been developed with WM164, WM35 and WM9 melanoma cells exposing them to sub-lethal concentrations of Dabrafenib at 25 nM, 10nM and 12 nM, respectively for about 12 days. IDTCs were then processed for flow cytometry to assess expression of various immune system regulators that may have a role in immune escape. Our preliminary study suggests that melanoma cells exposed to different drug concentration express the specific IDTC marker CD271. After initial screening of 12 different immune regulators, expression of Galectin-9 and CD276 was consistently down-regulated in different melanoma IDTCs compared to parental cells. TIM4 expression was down-regulated only in WM164 and WM9 in the drug resistant cells. We further aim to evaluate the functional role of these proteins in IDTC development and immune escape during cancer therapy.


Rab27a regulates invadopodia activity and invasion in melanoma

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The Rab GTPase family of trafficking proteins is increasingly being implicated in cancer cell biology. Genetic screens in early passage melanoma cell lines have identified Rab27a as a tumour dependency gene. While the role of Rab27a in melanosome trafficking in melanocytes is well known, its precise function in melanoma cells remains poorly understood.

In the present study, we found that Rab27a expression was significantly increased in human melanoma samples compared to benign nevi, and high Rab27a expression was associated with a poorer clinical outcome. Knockdown of Rab27a gene expression in melanoma cell lines inhibited invasion in a 3D spheroid assay, reduced cell speed in a collagen matrix, and reduced invadopodia activity in a fluorescent-gelatin degradation assay. Besides melanosomes, exogenously expressed Rab27a-GFP also partially co-localized with matrix metalloproteinase-14 (MMP14), which mediates matrix degradation by invadopodia. Rab27a knockdown reduced MMP14-positive actin foci in melanoma cells, suggesting a role for Rab27a in trafficking MMP14 to invadopodia and facilitating matrix degradation. Preliminary data also showed that a novel pan-Rab inhibitor could reduce the invasion of 3D melanoma spheroids and cell speed in collagen. We have also confirmed the previously reported role of Rab27a in promoting melanoma proliferation, with knockdown cells showing reduced proliferation.

These studies indicate that Rab GTPases, and Rab27a in particular, play a central role in mediating invadopodia activity and invasion in melanoma cells, making Rab27a a novel potential therapeutic target for inhibiting melanoma proliferation and metastasis.

Combination of sorafenib with aspirin synergistically target mutant NRAS melanoma

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The majority of melanomas are driven by activating mutations in the RAS-RAF pathway with around 40% of them being BRAF mutated and another 20% with mutant NRAS. Whereas BRAF-MEK inhibitors are highly effective in mutant BRAF melanoma patients, no clinically approved treatments are available for mutant NRAS melanoma patients. Here we report a novel strategy to target mutant NRAS melanoma by combining the multi-kinase inhibitor Sorafenib and the non-steroidal-anti-inflammatory drug acetylsalicylic acid (Aspirin). Combining Aspirin, but not the COX inhibitors Ibuprofen or Celecoxib significantly increased the in-vitro cytotoxicity of Sorafenib in mutant NRAS melanoma cell lines suggesting a COX independent mechanism. Mechanistically, combined exposure of Sorafenib and aspirin resulted in the simultaneous hyper-activation of the AMPK and ERK pathways. Combining Sorafenib with sole AMPK activators like Metformin or A769662 was not sufficient to induce cell death indicating that both ERK and AMPK activation are necessary for the observed effects. The combination was found to be specific for RAS mutant cells and had no significant effect in RAS wild type keratinocytes or melanoma cells. Currently an in vivo trial is running in mouse xenografts determining the applicability of the combination for mutant NRAS melanoma patients. The presented data show that the unique combination of Sorafenib and Aspirin might represent an alternative strategy to target RAS mutant cancers.

When two become one - A rare case of cutaneous sarcomatoid carcinoma

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Sarcomatoid carcinoma is a rare biphasic tumour displaying a dual epithelial and sarcomatous phenotype histologically, that is most commonly seen in the lung. We present an extremely rare case of a cutaneous sarcomatoid carcinoma on the left breast of a 30 year old female who had been treated for Ewing Sarcoma approximately 15 years prior. Whilst concluded to be a primary neoplasm, this case raises the question as to whether the two entities are related, and is there an underlying genetic predisposition.

An interesting asymmetry. A case of hidradenitis suppuritiva only in the affected leg of a young man with Klippel-Trenaunay syndrome

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Klippel-Trenaunay-Weber Syndrome is a rare disorder characterized by a triad of port-wine stain, varicose veings, and bony and soft tissue hypertrophy involving an extremity. We present the case of a 30 year old man with Klippel-Trenaunary Syndrome affecting the right leg, and a concurrent diagnosis of Hidradenitis Suppuritiva in this site only. To our knowledge, a case of Hidradenitis Suppuritiva of this pattern/association has not yet been documented in the literature, and presents an insight into potential aetiological factors of this debilitating chronic condition.

The effects of aging on intracellular lysosomal degradation in epidermal keratinocytes and dermal fibroblasts

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Fragmented extracellular matrix (ECM) induced by ultraviolet exposure and oxidative damage cause dermal elasticity decrease and epidermal turnover disorder. Epidermal keratinocytes and dermal fibroblasts have ability of endocytosis and degradation of the fragments. The degraded fragments are then recycled to form fresh ECM. Lysosomal activity is playing an important role in intracellular degradation systems. Lysosomes contain ECM-degradative enzymes which activate under acidic conditions. However, the relationship of lysosomal degradation and the production of fresh ECM to aging is currently unknown. Therefore, the present study examines the effects of aging on lysosomal degradation and whether aging influences the production of fresh ECM in epidermal keratinocytes and dermal fibroblasts. The results indicate that, along with aging, the activity of ECM degradative enzymes decreases due to loss of acidic conditions within the lysosomes. Inhibition of cellular lysosomal degradation leads to a decrease in fresh ECM production. These results suggest that age-related decrease in lysosomal degradation cause accumulation of fragmented ECM and decrease of fresh ECM production accelerate skin aging.

Standard melanoma-associated markers do not identify the MM127 metastatic melanoma cell line

Parvathi Haridas, Jacqui McGovern, Abhishek Kashyap, D.L. Sean McElwain, Matthew Simpson

Melanoma is an aggressive form of skin cancer that has the highest incidence rate in Australia. Since many aspects of melanoma research rely on the use of various types of melanoma cell lines, the reliable identification of different melanoma cell lines is very important. A range of melanoma-associated markers are used to identify different types of melanoma cell lines. A common feature of many experimental investigations is that some melanoma cell lines are unable to be detected using certain markers. To
address this limitation, many studies use two different markers to ensure reliable identification. The three most frequently used markers are: S100; HMB-45 and Melan-A. We explore the expression of these three markers in four different melanoma cell lines: WM35; WM793; SK-MEL-28; and MM127. The expression of these markers is examined at both the mRNA and protein level. Our results show that the metastatic cell line, MM127, cannot be detected using any of the commonly used melanoma-associated markers. This implies that it would be very difficult to identify this particular cell line in a heterogeneous sample, and as a result this cell line should be used with care.

**A romantic walk’s uninvited guest: A couple’s case of cutaneous lava migrans**

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A husband (37 years old) and wife (36 years old) presented with intensely pruritic areas on both feet 2 weeks after returning from a Thailand holiday. They had had a romantic barefoot beach walk during their holiday. No response was seen with topical terbinafine prescribed by their GP.

Clinically, the husband had an erythematous scaley annular eruptions with a serpiginous edge studded with occasional pustulovesicles. They extended from the second and third left toe web spaces bilaterally. No puncture wound noted. The wife had erythema and possible puncture site on lateral aspect of right second toe. There were also two separate erythematous serpiginous areas: on lateral aspect of border of foot and mid anterior tibia.

A clinical diagnosis of cutaneous lava migrans was made. Both patients received oral Ivermectin 12mg as a single dose and topical Betamethasone valerate cream for symptom relief.

Cutaneous lava migrans is caused by human infection with animal hookworm larvae, *Ancylostoma braziliense* or *Ancylostoma caninum.*[i] Humans become infected when filariform larvae in the soil penetrate the epidermis. The larvae cannot mature within the human host. Instead they migrate within the epidermis producing a localised inflammatory reaction, which may continue for weeks.[ii]


**Don’t brush off contact allergies in cheilitis: A case of toothbrush contact dermatitis**

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A 68 year old lady presented with a several year history of intermittent issues with her lips which escalated to become constant over the past six to twelve months. She describes lip dryness and cracking especially in both cornices of the mouth. It was associated with mild lip swelling several days a week. She denied systemisation of symptoms. She had a history of nickel allergy confirmed with patch testing. Her lips improved with topical methylprednisolone aceponate ointment and petroleum jelly as barrier ointment. It was exacerbated by chilli, curry, wind and possibly coffee.

Clinically, there was mild general dryness and slight scale of the lips with a focus of mild erythema in the cornices of the mouth. An irritant or allergic contact dermatitis was suspected and patch testing arranged.
The patient was patch tested with the common chelitis and toothpaste set from Contact Allergen Bank Australia and standard True Test Panel 1 and 2.

First patch test reading after 72 hours demonstrated Nickel ++, Mercapto-benzothiazole ++, Mercapto mix+ and Thiomersal +/- . Second patch test reading 48 hours later demonstrated Nickel +++ , Mercapto-benzothiazole ++, Mercapto mix+, Thiomersal + and Palladium chloride +.

Discussion with the patient identified her toothbrush as having multiple rubber strips attached to the head and shaft of brush. The patient changed to a simple toothbrush without rubber features and her cheilitis settled.

This case highlights that allergic contact dermatitis to rubber components need to be considered in cheilitis patients because increasingly modern toothbrushes contain rubber elements.

Cleaved CD147 shed from the surface of malignant melanoma cells activates MMP2 produced by fibroblasts

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Cluster of differentiation 147 (CD147)/basigin on the malignant tumor cell surface is critical for tumor proliferation, invasiveness, metastasis, and angiogenesis. CD147 expressed on malignant melanoma cells can induce tumor cell invasion by stimulating the production of matrix metalloproteinases (MMPs) by surrounding fibroblasts. Membrane vesicles, microvesicles and exosomes have attracted attention, as vehicles of functional molecules and their association with CD147 has been reported. Cleaved CD147 fragments released from tumor cells were reported to interact with fibroblasts. We investigated the intercellular mechanisms by which CD147 stimulates fibroblasts to induce MMP2 activity. Materials and Methods: CD147 was knockeddown using short hairpin RNA (shRNA). The stimulatory effect of CD147 in cell culture supernatants, microvesicles, and exosomes was examined by gelatin zymography. Results: Supernatants from A375 control cells induced increased enzymatic activity of fibroblasts; such activity was significantly lower in CD147 knock-down cells. Conclusion: Cleaved CD147 plays a pivotal role in stimulating fibroblasts to induce MMP2 activity.

Analysis of acute UVB reaction on basement membrane of the skin using a 3-D cultured human skin model.

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Background: UV causes various effects on human skin, such as acute sunburn reaction, photoaging and skin cancer induction. Previous studies exploring the pathogenesis of these effects have been primarily focused on changes in the epidermis and dermis, but the effect of UV to basement membrane of the skin has not been elucidated yet.

Objectives: We sought to evaluate the post-UVB acute reaction on skin basement membrane.
Methods: We assessed the condition of basement membrane before and after UVB irradiation (50 - 200mJ/cm²) using a 3-dimensional cultured human skin model (Kurabo Japan). The effects of UVB to basement membrane were analyzed immunohistochemically and by a western blot using antibodies to various skin basement membrane components.

Results: The expressions of basement membrane components (nidogen1, laminin, type IV collagen) were diminished and the epidermis-dermis junction were disturbed at day 2 and 4 after low dose UVB irradiation. After high dose UVB irradiation, interstitium formation at the epidermis-dermis boundary was caused immediately and no signal of basement membrane components were detected until the 4th day.

Conclusions: We first demonstrated that low dose UVB irradiation decreased basement membrane components in addition to the epidermal tissue damage in acute phase histologically and biochemically. As we expected, the higher dose of UVB exposure caused more serious damage. These results suggest that deteriorating changes occur not only in epidermis but also in the basement membrane in the acute phase after UVB exposure, which is related to the blister and erosion formation at the time of sunburn.

Cutaneous adverse events of immunotherapies anti-melanoma: Classification and management

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Over the past five years, immunotherapies such as anti-Cytotoxic T lymphocyte antigen-4 (anti-CTLA4) and anti-Programmed cell Death 1 (anti-PD1) antibodies have improved treatment responses and overall survival in patients with advanced melanoma. With increased use of these therapies, a range of cutaneous toxicities have emerged, varying from rare but serious adverse events such as toxic epidermal necrolysis, and drug reaction with eosinophilia to frequent but often mild lichenoid reaction. Patients receiving ipilimumab commonly develop maculopapular exanthema (as frequent as 68%), pruritus (31%), vitiligo (11%); whilst patients on anti-PD1 antibody often develop lichenoid reaction (17%), vitiligo (24%), pruritus (12%) and vesiculo-bullous reactions such as bullous pemphigoid. Early detection and management of these cutaneous toxicities will aid patients to receive appropriate treatments, avoid unnecessary discontinuation of anti-tumour therapies and improve the patient’s overall quality of life.

Two cases of advanced cutaneous squamous cell carcinoma successfully treated by combination therapy with cetuximab and paclitaxel

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Cetuximab is a monoclonal antibody that binds to epidermal growth factor receptor. Recently, combination therapy with cetuximab and paclitaxel has been used as a standard therapy for squamous cell carcinoma (SCC) of the head and neck. We herein report two cases of advanced SCC of the skin successfully treated with the combination of cetuximab and paclitaxel. The first case was a 53-year-old female presenting with a large tumor of the vulva. She was diagnosed with vulvar SCC with vaginal invasion, and multiple metastases to the inguinal lymph nodes. She was treated with 5-fluorouracil and cisplatin, peplomycin, radiotherapy, and irinotecan. However, the tumor was resistant to these therapy and regrew. We initiated cetuximab (initial dose of 400 mg/m2, followed by 50 mg/m2) and paclitaxel (80 mg/m2) , on a weekly basis. After ten cycles of the treatment, the serum SCC antigen level had decreased from 33.7 ng/mL to 2.6 ng/mL, and the tumor of the vulva had regressed. The second case was a 52-year-old female presenting with a large SCC of the buttock arising from perianal chronic pyoderma.
She also developed multiple metastases to the inguinal and intra-abdominal lymph nodes. She received chemotherapy and radiotherapy after tumor debulking, however, the tumor was resistant to these therapy. Following six cycles of cetuximab and paclitaxel treatment, the serum SCC antigen level had decreased from 66.9 ng/mL to 12.0 ng/mL, and the lymph nodes metastases regressed. Cetuximab and paclitaxel should be considered as second therapy for advanced SCC of the skin.

**Bowenoid papulosis in chronic immunosuppression**

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A 38-year-old Iranian refugee with a history of chronic immunosuppression presented for a review of biopsy proven penile Bowenoid papulosis. Cryotherapy was ineffective and the lesions gradually increased in size over the past eighteen months. He now reported a new verrucous growth on his left thumb that was intermittently painful and pruritic. Past medical history was significant for myelodysplastic syndrome, disseminated MAC infection (treated and cleared) and an open splenectomy. Concurrent medications included rifampicin 600mg daily and azithromycin 500mg daily. Physical examination revealed multiple, pigmented blackish brown papules with a flat- to verrucous surface in the suprapubic region (Panel A). A verrucous growth was noted on his left thumb without any underlying nail changes (Panel B). Imiquimod 5% cream was prescribed to apply thrice weekly to the affected areas. After 6 weeks of follow-up he noted mild improvement in his penile lesions and decreased pruritus of the left thumb.

Bowenoid papulosis is a rare clinical entity characterized by numerous, flat, hyperpigmented or violaceous papules commonly affecting the genitalia of young adults. It is strongly associated with human papilloma virus (HPV) and usually affects sexually active adults with a slight female predominance. Chronic immunosuppression for various reasons is a significant risk factor for persistent infections with HPV and HPV-associated disease. The increased prevalence of HPV induced lesions in individuals with a range of immunodeficiencies, primary or acquired, emphasizes the significance of adaptive and cell mediated immunity in the control of symptomatic HPV infections.


Two cases of scarring alopecia

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We report herein two cases of scarring alopecia in patients with discoid lupus erythematosus (DLE) and psoriasis, and respectively describe the clinical and histological features.

Case 1: A 41-year-old Japanese woman presented with a 4-year history of progressive hair loss on her scalp and with a 10-year history of DLE involving her face. Clinical examination revealed sharply demarcated and slightly indurated erythematous plaques on the face and a well demarcated round and reddish - purplish macules with scaling into the patchy scarring alopecia. Histopathologically, the epidermis showed lost of rate ridge patterns, basal vacuolar degeneration and hyperkeratosis with follicular plugging in stratum corneum. Lymphocytic infiltrate was observed around hair follicles and other appendages. Based on these findings, scarring alopecia in DLE was made.

Case 2: A 59-year-old Japanese woman consulted us for a patchy alopecia of the scalp that had appeared 9 years previously. Clinical examination revealed that large and thick scaling plaques were present on the vertex area with patchy cicatricial alopecia, and red scaly distributed small plaques on her trunk and buttocks were also present. Histopathologically, increased the fibrosing of dermis, partial lack of hair follicles, and sebaceous glands and perifollicular lymphocytic infiltration were observed in the alopecia lesion. Histopathology of the skin lesion revealed typical psoriatic findings. Based on these findings, scarring alopecia in psoriasis was made.

Clinical and histopathological features with DLE and psoriasis are demonstrated to establish its unique status among the disorders of scarring alopecia.

Pemphigoid without mucosal involvement showing autoantibodies against laminin-332 alpha3 subunit

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Autoimmune response to laminin-332 results in development of mucous membrane pemphigoid. Herein, we report a unique case of bullous disorder without mucosal involvement in the presence of IgG autoantibodies to the alpha3 subunit of laminin-332. A 60-year-old Japanese man was referred to our department with a 2-week history of erythema and bulla. Clinical examination revealed itchy tense blisters and erythema located predominantly on the trunk and extremities. No mucosal lesions were observed. Histopathological examination of the lesion on the abdomen revealed a subepidermal blister with infiltration of neutrophils. Direct immunofluorescence revealed linear deposition of IgG and C3, but not of IgA, along the BMZ. Indirect immunofluorescence revealed circulating IgG anti-BMZ autoantibodies that bound to the dermal side of salt-split normal human skin. ELISAs showed negative results for BP180, BP230 and type VII collagen. In addition, immunoblotting with purified human laminin-332 revealed that patient IgG antibodies bound to the 165-kDa, 145-kDa protein, corresponding to the alpha3 subunit, although the other immunoblotting were all negative. Prednisolone (30 mg daily) alone or in combination with dapsone (75 mg daily) partially suppressed the development of new blisters over several months. During the course, esophageal carcinoma has been detected in the patient after 1 year.
Real-time cell cycle and cell death imaging of the effect of sphingosine kinase inhibition on 3D melanoma spheroids

Arne Kienzle, Eileen McGowan, Garth Douglas, Dominik Kaczorowski, Wolfgang Weninger, Nikolas K Haass

Preclinical in vitro studies often poorly predict the outcome of clinical studies. Thus, we use 3D melanoma spheroids which mimic tumour architecture and microenvironment better. Using the fluorescent ubiquitination-based cell cycle indicator (FUCCI) system combined with DRAQ7 staining allowed us to conduct real-time cell cycle and cell death analysis in 2D and 3D culture.

Inhibition of sphingosine kinase 1 (SK1) is a promising approach for treating melanoma. SK1 plays a critical role in determining the dynamic balance between the proapoptotic sphingolipid metabolite ceramide and the prosurvival sphingosine-1-phosphate. SK1 expression was confirmed in 29 melanoma lines by microarray, qPCR and immunoblotting. SK1 functionality was proven by enzyme activity assays. Melanoma growth reduction was achieved through inhibition of different stations within the SK1 pathway. Fingolimod (FTY720), dimethyl-sphingosine (DMS) and sphingosine kinase inhibitors 1 and 2 (SKI-1, SKI-2) decreased cell viability and caused cytotoxicity. Interestingly, whereas DMS and SKI-1 caused G1-arrest, FTY720 and SKI-2 caused G2-arrest. However, in neither case the arrest was as profound as in the positive G1-arrest control (MEK inhibitor U0126), indicating that cell death in the SK1-inhibited cells is primarily independent on a specific cell cycle phase.

These findings are important as different subpopulations of 3D spheroids and in vivo show different cell cycle behaviour and respond differently to drugs. While when using conventional methods the effect of SK1 inhibition would appear superficially very similar to that of MEK inhibition, our model allows the investigation of subtle differences in mechanism of action in real time and in 3D.

Targeting Cancer: Antibody-functionalized Dendritic Mesoporous Silica Nanoparticles as a novel, pH-sensitive and targeted Drug Delivery System to encapsulate and transport TNF-α

Arne Kienzle, Sven Kurch, Janine Schlöder, Nikolas K Haass, Wolfgang Tremel, Helmut Jonuleit

Aims: TNF-α is one of the most well-known anti-tumour factors. Due to its cytotoxic effect on tumour cells and its pro-inflammatory potential, TNF-α has the ability to slow tumour growth. However, its pharmacological use is significantly limited by its high systemic toxicity. Therefore, we synthesized a drug delivery system combining TNF-α loaded, dye functionalized dendritic mesoporous silica nanoparticle (DMSN) with a pH-sensitive PEI-PEG copolymer for pore coverage. The major objective of this study is the transport of TNF-α in a nontoxic carrier system followed by a tumour tissue specific release to induce anti-tumour activity without causing systemic toxicity.

Methods: MTT assays were used to analyze biological activity of encapsulated TNF-α. Solubility and functionalization of the DMSN were monitored by zeta potential, dynamic light scattering and transmission electron microscopy measurements. Following the functionalization with anti-EGFR-antibody, which was controlled by FACS, DMSN will be used in humanized, tumour-bearing NOD/Scid1gHLA-A2+ mice.

Results: Encapsulated TNF-α showed a 97% reduced toxicity compared to free TNF-α after 12h of treatment. TEM, DLS and zeta potential demonstrated stable and functional particles under normal culture conditions. FACS data demonstrated successful anti-EGFR conjugation to the particles PEI-PEG copolymer.

Conclusion: Here we demonstrate the combination of DMSN for transport and pH-sensitive PEI-PEG copolymer for pore coverage as a potent system allowing the transport of toxic anti-tumour biologicals. Our promising preliminary results allow further investigation, using anti-EGFR functionalized DMSN to limit the systemic toxic effects of TNF-α and target cancer in vivo.
The third case of digenic inheritance in KRT5 and KRT14 in epidermolysis bullosa simplex and 9 other novel keratin mutations in the Australian EB population

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Epidermolysis Bullosa Simplex (EBS) is a rare heritable skin fragility disorder, most commonly caused by dominant mutations in the either of the genes encoding keratin 5 (KRT5) and keratin 14 (KRT14). EBS shows clinical heterogeneity with localised, intermediate and generalised-severe forms, which tend to correlate with the location and nature of the mutation. Autosomal recessive inheritance has also been reported.

There have been two cases reported where digenic inheritance was observed, whereby variants in two different genes, when combined, result in a different phenotype. The first case was described in Israel, where the proband with intermediate form of EBS inherited Ile183Thr in KRT5 maternally, who was mildly affected with a EBS-localised, and Arg388His in KRT14 from his unaffected father. A similar second case was described in Poland where Arg471His in KRT5 inherited from the unaffected mother and Met272Thr inherited from the mildly affected father resulted in an intermediate severity of EBS in the proband.

We report the third case of such rare digenic inheritance in EBS. The proband was affected with a EBS-localised inherited Met294Thr in KRT14 paternally and Leu155Pro in KRT5 maternally. Unlike the two previous digenic cases, neither parent was affected with EBS. This supports the theory that the EBS phenotype is modulated by biallelic mutations. Including this case, we have found 31 different mutations in the KRT5 and KRT14, 11 of which were novel. The genotype-phenotype correlation seen in the Australian EBS population highlights the importance of the location and nature of KRT5 and KRT14 mutation in determining the disease severity.

A case report of psoriatic arthritis with congenital adrenal hypoplasia successfully treated with infliximab

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Psoriatic arthritis (PsA) is an inflammatory arthritis associated with psoriasis. It has become clear that the levels of tumor necrosis factor alpha (TNF-α) are elevated in both blood and lesional skin in patients with PsA. Past studies have shown that glucocorticoids suppress TNF-α expression by effecting on gene transcription and post-translational regulation. Although the efficacy and safety of biologic treatments have been well established in patients with PsA, there have been no reports on biologic therapy for patients with PsA complicated by congenital adrenal hypoplasia (AHC), which is a rare disorder of adrenocortical insufficiency. To our knowledge, this is the first reported case of PsA with AHC improved with infliximab. A 21-year-old Japanese female with a 5-year history of psoriasis vulgaris was referred to us. She also had swollen toes and arthralgia in the knees for one year. Notably, she had been diagnosed at birth with AHC on the basis of tests for adrenal function, which had resulted in a low baseline cortisol level. After appropriate clinical and laboratory evaluation, we diagnosed her as having an active PsA with AHC. She started on infliximab at 5 mg/kg in combination of 4 mg/week methotrexate. With the infliximab treatment, she achieved an ACR70 response and a PASI-100 response within 6 months. She received infliximab treatment for more than a year without any adverse effects. Although a long-term follow-up is required to clarify the safety of treatment of infliximab in our case, TNF-α blocker should be considered for PsA patients with AHC.
Skin wound inflammation modulated by IL-17 producing macrophages is dependent on IL-23

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Inflammation is a critical phase in the healing of skin wounds. Excessive inflammation and inflammatory macrophages are known to cause impaired wound closure and outcome. We previously reported IL-17 as a major differentially expressed cytokine between inflammatory macrophages and their alternatively activated counterparts. Given that IL-23 is an upstream regulator of IL-17 expression in T-helper cells, we hypothesized a major role for IL-23 in macrophage IL-17 expression and in their polarisation in wounds.

IL-23 but not IL-12 deficient mice displayed significantly reduced IL-17 expression in wound macrophages. This was rescued by delivery of recombinant IL-23. IL-23 deficient mice showed a significant increase in non-inflammatory macrophages. In accordance, addition of IL-23 strongly amplified the inflammatory phenotype of macrophages in vitro. These changes in macrophage polarisation through defective IL-17 expression influenced wound proliferation and closure in obese diabetic mice, through the reduction of iNOS expression. This study highlights the importance of the IL-23 and IL-17 pathway in wound macrophage polarisation, offering new possibilities of therapeutic intervention in chronic wounds.

Establishment of keratinocytes derived transgene-free induced pluripotent stem cells from recessive dystrophic epidermolysis bullosa patients

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Lentivirus- and retrovirus-derived induced pluripotent stem cells (iPSCs) from recessive dystrophic epidermolysis bullosa (RDEB) patients have been reported. Conversely, transgene-free methods have not been investigated for generating iPSCs from RDEB keratinocytes. Several transgene-free methods have been recently reported for establishing iPSCs, such as Sendai viral vectors (SVVs), to prevent tumorigenesis. In this study, we established transgene-free iPSCs derived from RDEB keratinocytes using SVVs. Keratinocytes were obtained from two patients with RDEB, generalized intermediate type. We transduced these keratinocytes with Oct3/4, Sox2, Klf4 and c-Myc by SVVs. Twenty days later, iPSC-like colonies were picked up and we continued further culturing. On passage 7, we confirmed the negativity of SVV-derived induction factors by RT-PCR, and then we used these keratinocyte-derived iPSCs (KiPSCs) for further analyses. The morphology of all the KiPSC lines appeared similar to those of human embryonic stem cells. These KiPSC colonies were identified by positive alkaline phosphatase staining, and the expression of stem cell markers was detected by immunohistochemical and RT-PCR analyses. Bisulfite sequencing revealed demethylated patterns in the NANOG promoter region, which indicates undifferentiation in KiPSCs. Embryoid body formation assays demonstrated that the KiPSCs were capable of differentiating into all three germ layers in vitro and we also confirmed the in vivo differentiation capabilities of our KiPSCs by teratoma formation. Furthermore, we proved that these KiPSCs can differentiate into keratinocytes and dermal fibroblasts. Our data demonstrate that we can generate SVV-driven transgene-free iPSCs from RDEB keratinocytes, which is a safe and less invasive reprogramming technique.
Naevus endophenotypes and melanoma risk

Seamus McWhirter, Katie Lee, Glen Wimberley, Natalie Ong, Anna Leikvold, Alastair Ashley, Kasturee Jagirdar, David Duffy, H. Peter Soyer, Richard Sturm

GWAS studies have reported that a number of SNPs are associated with total naevus count, which is almost 70% heritable and known to be the most significant risk factor for melanoma development. Five loci MTAP, PLA2G6, IRF4, TYR and MITF are known to influence naevus count and melanoma risk, however other naevus associated traits are yet to be tested for an association with these genes. The aim of this study was to test the association between the dermoscopic pigment pattern of naevi and these five naevus genes, with the view to expand the endophenotype of these genes to include other characteristics and behaviors of naevi. This has been examined in 1026 melanoma cases and controls living in Southeast Queensland in the Brisbane Naevus Morphology Study (BNMS). Subjects have total naevi counts and pigmentation phenotype data recorded, along with dermoscopic imaging of all naevi >5mm not covered by underwear. Dermoscopic images of naevi have been classified according to either of 2 independent scoring systems, and then tallied into subclasses to make inferences as to association with these known naevus genes. The correlation of these 2 reading systems has been tested by comparing the classification all naevi from a subset of 260 individuals using both methods. With dermoscopy being the primary tool used by Dermatologists to aid in differentiating between benign and malignant lesions, it is thought that correlating naevus and melanoma genes to common dermoscopic naevus patterns will also further the clinical relevance of the practice of dermoscopy.

Anaplastic large cell lymphoma involving skin and muscle associated with polymyositis

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There is a well-documented association between autoimmune inflammatory myopathies and several malignancies. Among hematologic malignancies, B-cell lymphomas are more commonly reported than T-cell lymphomas. Here we report a rare case of anaplastic large cell lymphoma (ALCL) involving skin and muscle associated with polymyositis. A 32-year-old Japanese man was referred to our hospital with muscle weakness with elevated serum creatine kinase (CK) levels. Muscle biopsy of the biceps revealed inflammatory mononuclear cell infiltration within the endomysium and muscle fiber necrosis and regeneration. He was diagnosed as having polymyositis. He had been treated with oral prednisolone, intravenous gamma globulin, and oral immunosuppressant agents such as cyclosporine A, tacrolimus, or methotrexate, and his symptoms had improved moderately. After four years, muscle weakness exacerbated without elevation of serum CK levels. Scattered erythematous plaques and nodules with occasional ulceration and several intramuscular nodules appeared. Biopsy specimens of both skin and intramuscular nodules revealed massive infiltration of medium- to large-sized atypical lymphocytes. They were positive for CD3 and CD30, and negative for CD20, CD56, or EBER. Monoclonal T cell receptor (TCR) rearrangement was detected in both skin and intramuscular nodules. As the same TCR rearrangement peak and infiltration of a small number of CD30-positive lymphocytes in muscle fiber were found in the first muscle biopsy specimen, we diagnosed him as having ALCL involving skin and muscle, which exacerbated after 4 years of immunosuppressive therapy. As muscle weakness continued in spite of cessation of oral methotrexate, he is now treated with CHOP therapy.
Increased serum levels of interleukin 21 in adult patients with atopic dermatitis

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Interleukin (IL)-21 is a member of the type I cytokine family and takes part in the pathogenesis of T helper type 2 allergic diseases. IL-21 expression was upregulated in acute skin lesions in atopic dermatitis (AD) patients, but the relationship of serum IL-21 with disease severity and laboratory markers in patients with AD remained unclear. The aim of this study was to quantify serum IL-21 levels in patients with AD and to evaluate the relationships of serum IL-21 levels with the disease severity, laboratory markers, and eruption types. We measured serum IL-21 levels in adult patients with AD and healthy control subjects by enzyme-linked immunosorbent assay. Serum levels of IL-21 were significantly higher in adult patients with AD compared with those in healthy control subjects. Evaluation of relationships of serum IL-21 with clinical severity revealed that IL-21 were significantly higher only in patients with severe AD, but not in patients with mild and moderate AD, compared with those in healthy control subjects. Serum IL-21 levels were not correlated with serum IgE, serum thymus and activation-related chemokine, blood eosinophilia, serum lactate dehydrogenase, and skin symptom severity scores. These results suggest that IL-21 may be involved in the development of severe AD.

Mycobacterium chelonae infection of the arm presenting in a renal transplant patient with history of calciphylaxis

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A renal transplant patient with a history of calciphylaxis presented with a 4 month history of tender suppurative nodules on the left arm. The patient was systemically well but the arm was swollen and painful. There was no history of injury to the arm. Incisional biopsy showed mycobacteria present on wade fite stains and culture grew mycobacterium chelonae. Calcification of vessels was seen throughout the biopsy consistent with the history of calciphylaxis. Treatment of calciphylaxis had been with renal transplantation and parathyroidectomy and symptoms had resolved but vessel changes persist on histology. The patient admitted to having applied an oil purchased on the internet to this arm which claimed to treat calciphylaxis. Cultures of the oil and containers it was supplied in are in progress.
Determination of the best objective clinical outcome measure for bullous pemphigoid

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Objectives: In order for clinical trials to proceed in a particular disease process, the objective measure for scoring the severity of that disease needs to be fully validated, including knowing what the minimal significant clinical change in that score is. This has yet to be achieved with the most common autoimmune blistering disease, bullous pemphigoid (BP). The aim was to evaluate the suitability of the disease specific Bullous Pemphigoid Disease Area Index (BPDAI) compared to the general Autoimmune Bullous Skin disorder Intensity Score (ABSIS) as outcome measures for bullous pemphigoid.

Methods: Thirty-two BP patients were repeatedly assessed over four years using Physician Global Assessment (PGA), anti-BP180 ELISA titres, BPDAI, ABSIS, BPDAI-Pruritus, Autoimmune Bullous Disease Quality of Life (ABQOL) and Treatment of Autoimmune Bullous Disease Quality of Life (TABQOL) questionnaires. The reliability, validity, responsiveness, and minimal clinically important differences (MCIDs) for the two objective scores (BPDAI and ABSIS) were calculated.

Results: For inter-rater reliability, the intraclass correlation coefficients (95% CI) were: BPDAI 0.957(0.901-0.982) and ABSIS 0.881(0.736-0.949). Compared to ABSIS, BPDAI was better correlated with PGA (r=0.875, p<0.001), BPDAI-Pruritus (r=0.632, p=0.004), ABQOL (r=0.521, p=0.011) and TABQOL (r=0.538, p=0.008). MCIDs for BPDAI are 4-points for assessing clinical improvement and 3-points for deterioration. MCIDs for ABSIS are larger at 8.6-points for improvement and 4-points for deterioration.

Conclusion: BPDAI demonstrated excellent reliability, validity and responsiveness, while ABSIS had moderate to good reliability, validity and responsiveness for bullous pemphigoid. The BPDAI is the most reliable clinical outcome measure for BP.

The risk of coronary artery disease in psoriasis is not associated with HLA-C*06:02

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Psoriasis vulgaris (PV) is a cutaneous inflammatory disease which is a combination genetic and environmental factors. A population survey of association between HLA-C antigens and susceptibility to PV with or without coronary artery diseases (CAD) was done, using 206 Japanese patients with PV (mean age 43.2 ±17.3 years).

Out of the 206 psoriatics, twelve male PV patients had CAD. Out of the 12 PV patients with CAD, 1 had mild PV (<3% body surface area (BSA)), 4 had moderate PV (3-10% BSA), and 7 had severe PV (10% <BSA). The association increases with increasing disease severity, based on the evaluation by BSA (p <0.001).

Phenotype frequency of HLA-C*06:02 in 206 patients with PV was significantly increased, as compared to those of the 163 healthy controls (9.7% in PV vs 0.6% in controls; pc <0.001, Odd’s ratio=11.91). However, we could not find a statistically significant association between HLA-C*06:02 and susceptibility to PV with CAD (p >0.6), although there was none having HLA-C*06:02 among the 12 PV patients with IHD. Thus, these results indicate that PV-susceptible allele, HLA-C*06:02, may be a different genetic factor concerning comorbidity of PV with CAD.
Supersaturation of calcipotriol and betamethasone dipropionate – facilitating the improved clinical efficacy of the fixed combination foam LEO90100 (calcipotriol 50 microgram/g (Cal) plus betamethasone dipropionate 500microgram/g (BD) as compared to other formulations of Cal/BD

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Calcipotriol (Cal)/betamethasone dipropionate (BD) foam was recently approved in the US and in Europe for the topical treatment of psoriasis. Cal/BD foam has demonstrated improved clinical efficacy compared to other formulations of Cal/BD1.

The propellant of Cal/BD foam that acts as a solvent for the active pharmaceutical ingredients (APIs), evaporates rapidly upon application, leaving fully dissolved supersaturated APIs available for skin penetration.

The properties of a topical formulation are important for patient preference as well as drug delivery. Skin penetration of the API is proportional to the concentration of dissolved API on the skin. Supersaturation of API is known to improve skin penetration 2.

A novel approach was undertaken to investigate if supersaturation offers an explanation for the enhanced efficacy seen with Cal/BD foam.

Microscopy (200x), X-Ray Powder Diffraction (XRPD) and Raman-imaging was used to identify any crystalline particles of Cal/BD in foam and ointment, when applied and 18 hours after application no Cal/BD crystals could be identified in the foam. Microscopy and Raman identified Cal/BD crystals in the ointment. XRPD identified BD but not Cal crystals in ointment.

This study documents that the enhanced local bioavailability of Cal/BD in the foam vehicle results from supersaturation, a potential explanation for the improved clinical efficacy observed in clinical trials.

1. 1.Paul et al; EADV 2015; Copenhagen, Denmark; Poster P1724
2. 2.Cilurzo et al; Curr Pharm Design 2015;21(20):2733-44

High burden of Merkel cell polyomavirus DNA in the sun-exposed skin of elder adults

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Merkel cell carcinoma (MCC), one of the most aggressive skin cancers, develops in sun-exposed skin of the elderly and is linked to infection with the Merkel cell polyomavirus (MCPyV) (Fege H, et al. Science 2008; 319: 1096-1100.). However, the association of ultraviolet light irradiation with the MCPyV infection in healthy individuals remains unclear. Here, we investigated the healthy sun-unexposed skin and sun-exposed skin for the MCPyV loads in healthy donors by comparing between young individuals and old individuals. (Methods) This study included 284 Japanese participants. Sun-unexposed arm and sun-exposed forehead skin swab samples were obtained and analyzed for MCPyV infection, using quantitative real-time PCR and the PCR products were subjected to DNA sequencing. (Results) MCPyV DNA levels were significantly higher in swabs obtained from the individuals aged >40 years compared with those aged 60 years. (Conclusion) Findings of this study suggested that MCPyV infection with high viral loads is prevalent
in the sun-exposed skin of elder individuals. Whether individuals with high MCPyV loads may preferentially develop MCC is an important question to be resolved.

A case of Lymphoepithelioma-like carcinoma of the skin

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Lymphoepithelioma-like carcinoma of the skin (LELCS) is a rare, low malignant cutaneous neoplasm. It is usually caused by sun exposure and is most prevalent on the skin of the head and neck in elderly individuals. There is a tendency toward local recurrence and very limited metastatic potential. Excision with a wide surgical margin is recommended. The histologic etiology of LELCS is still unclear. We present a case of a 57-year-old Japanese female with an asymptomatic red nodule on her left lower eyelid. Diagnosed with seborrheic keratosis, the tumor was resected by electrocauterization. Three months later, the tumor reappeared and was excised marginally as nevus cell nevus versus basal cell carcinoma or adnexal tumor. She did not have any palpable lymphadenopathy. Histopathologically, the entire dermis was occupied by multiple lobules of atypical epithelial tumor cell nests lacking connections with epidermis. The nests were surrounded by marked lymphocytic inflammatory cells. Epithelial tumor cells presented a rich amount of eosinophilic cytoplasm and large, prominent nuclei. Immunohistochemical examination showed that the epithelial tumor cells were positive for AE1/3 and S-100. The inflammatory cells were positive for CD3 and CD79a. Because of these findings, the present case was diagnosed as LELCS. We carried out wide local excision after excluding any other primary lesion or metastasis by nasopharyngoscopy and positron emission tomography. She has not been detected recurrence or metastasis in about five years observation after the operation.

Efficacy of smartphone applications in detecting melanoma

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There currently exist smartphone apps which aim to autonomously risk stratify melanocytic lesions using a smartphones inbuilt camera, so called “melanoma apps”. These applications do not require clinician input nor use dermoscopic attachments though their efficacy remains unclear. This study aimed to determine the utility of autonomous smartphone applications in detecting “high risk” melanocytic lesions and confirming the diagnosis of benign naevi. 3 autonomous risk stratification applications were identified on Android or iOS (January 2015). Patients (n=37) were recruited and a clinical trial of the applications ability to risk stratify 62 pigmented lesions recruited (n=62) was conducted. The risk stratification of the pigmented lesion provided by the applications was compared against the dermatologist’s diagnosis of risk. Lesions being monitored long term were “clinically benign” and those sent for excision “clinically suspicious”. Preliminary results and analysis will be presented.
The efficiency of steroid pulse therapy in patients with severe alopecia areata in Kurume University

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The corticosteroid pulse therapy for alopecia areata (AA) is widely reported. We evaluated the efficiency and safety of steroid pulse therapy for severe alopecia areata. Of total 26 AA patients aged from 16 to 66, 6 male and 20 female were included in this study. Among the patients, 24 (92%) had >50% hair loss area of the scalp. All patients except two patients were treated with 1000mg methylprednisolone by intravenous administration. Among 26 AA patients, 15 patients were administrated steroid pulse therapy within 3 months of onset, 3 patients were administrated within 6 months of onset, and 5 patients were administrated within twelve months of onset. 3 patients were administrated after twelve months of onset. In patients group administrated within 3 months of onset, 80% was good responders (>70% improvement), and one patient within 6 months of onset and 2 patients within twelve months of onset were good responders. In contrast, the patients group administrated after twelve months of onset, no patient was good responder.

Incidence and prevalence of non-melanoma skin cancer in Australia: A systematic review

Eshini Perera, Neiraja Gnaneswaran, Carolyn Staines, Aung Ko Win, Rodney Sinclair

Purpose: Non-melanoma skin cancer (NMSC), including basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), is the most common cancer occurring in people with fair skin. Australia has been reported to have the highest incidence of NMSC in the world. The lack of a nationwide cancer registry data has been a barrier to conducting population based research on these cancers.

Methodology: Using a systematic search of the literature, we identified 21 studies, beween 1948 and 2011, that investigated the incidence and prevalence of NMSC in Australia.

Results: There were 6 studies that were conducted on national-level, two at state-level and 13 at the regional-level. Overall, incidence of NMSC had steadily increased over calendar-years in Australia. The incidence of NMSC per 100 000 person-years was estimated to be 555 in 1985; 977 in 1990; 1109 in 1995; 1170 in 2002 and 2448 in 2011. The incidence was higher for men than women and higher for BCC than SCC. Incidence varied across the states of Australia, with the highest in Queensland. The prevalence of NMSC was estimated to be 2% in Australia in 2002.

Conclusion: This literature review is the most comprehensive review to date investigating the incidence and prevalence of NMSC in Australia. The incidence and prevalence of NMSC needs to be accurately established at both national and state levels to determine the costs and burden of the disease on the public health system in Australia. However, a cost-effective and practical method of documenting NMSC cases may be scope for further research.
Higher rates of re-excision in smaller NMSC specimens – Does initial tumour size predict rates of residual disease?

Eshini Perera, Neiraja Gnaneswaran, Rodney Sinclair

Purpose: Identification of key risk-factors for re-excision of NMSC may assist operative planning or early referrals to specialist centres. The aim of this study was to identify the size-distribution of the original NMSC that required treatment for residual NMSC.

Methodology: Medicare item-numbers for treatment of NMSC were pooled and frequencies of each item-number were resolved by gender and age. These item-numbers were separated into item-numbers for primary, recurrent and residual treatment. Residual treatment for NMSC was examined with respect to size of initial lesion (<10mm, 11-20mm, >20mm).

Results: 11,594 of original NMSC lesions requiring a second treatment for residual occurrence were under 10mm, representing >50% of the residual NMSC billed. Original NMSC lesions >20mm were less likely to require treatment for residual NMSC, with only 8.6% of all residuals billed having an original NMSC lesion over 20mm. 55% of residual lesions that were originally < than 10mm in size were treated by the same practitioner. 8.7% of lesions that were originally >20mm were treated by another practitioner. Original lesions that were <10mm were excised by a GP in 54% of the cases, while original lesions that were over 20mm were excised by specialists in 64% of cases.

Conclusion: These results demonstrate that a large burden of recurrent disease on specialist centres exists. Treatment by non-excisional methods accounted for a large proportion of the recurrent disease. This suggests that, non-excisional methods have greater risks of recurrence. Clearer guidelines for referral as well as greater availability to specialist centres would help target this burden of disease.

A Case of ‘Polymyalgia Rheumatica like disease’ following Brodalumab treatment for moderate to severe chronic plaque psoriasis

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Brodalumab is a human monoclonal antibody that targets interleukin 17 receptor A. Brodalumab has been trialed for treatment of chronic plaque psoriasis and limited published data exists reporting possible adverse effects upon biologic cessation. We present a rare case of polymyalgia like illness occurring post brodalumab treatment in a young male. This case provides insight that may help reveal the long standing unknown aetiology of polymyalgia rheumatica (PMR).
Think length of sebum suppression, not cumulative dose, when using isotretinoin for acne

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Some isotretinoin guidelines recommend cumulative doses of 120-140 mg/kg. This arose from a mathematical calculation of the daily dose (1 mg/kg/day), taken for a fixed 16-weeks. This time period was a compromise in an attempt to minimise the teratogenetic exposure to women; it was not based on any science.

Much lower dosages of isotretinoin (eg, 0.05 mg/kg/day) are equally effective, both in speed of response and total clearance of acne. However patients treated with very-low daily dosages for only 16-weeks, may relapse earlier. Unfortunately this has been incorrectly interpreted as being a reflection of cumulative dose.

We now appreciate that higher dose isotretinoin (0.5-1.0 mg/kg/day) has a prolonged effect on sebum suppression, through apoptosis of both sebaceous gland cells and their stem cells; this persists for 4-8 months after discontinuation. This effect on stem cells is not seen with very-low dose (0.05-0.1 mg/kg/day), so the sebum suppression only lasts for another 4-8 weeks after stopping isotretinoin.

We reviewed 1453 acne patients treated with isotretinoin for the first time: 326 (22.4%) relapsed requiring a 2nd course. Relapers were more likely to be women (61% v 47%, p<0.001), have received a larger daily dose (0.71 mg/kg/day v 0.58 mg/kg/day, p<0.001) and greater cumulative dose (126 mg/kg v 101 mg/kg, p<0.001).

Neither daily nor cumulative dose influences relapse of acne. The length of sebum suppression is more important.

Dual proliferation behaviour of epidermal progenitors

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The maintenance of the interfollicular epidermis (IFE) remains a controversial subject. Conflicting studies support the existence of a stem cell hierarchy in the IFE or a committed progenitor model of random fate choice between self renewal and differentiation without hierarchy. To allow fate tracking of rare stem cell populations, we performed a high density multicolour labelling of all basal keratinocytes using rainbow technology and followed their fate over time.

Epidermal clones within the IFE of dorsal skin grew in size from 1 week to 6 months after labelling (p<0.0001) in accordance with the committed progenitor. However, clones connected to hair follicles were larger (p<0.05) and grew more steadily over time (p<0.0001). Further investigations showed that clones in areas of active hair cycling were larger compared to clones not attached to HF (p<0.05) and that proliferation in the HF infundibulum occurred mainly in anagen (p<0.0001) suggesting that this hair cycle phase promoted the growth of IFE clones.

Despite the increase in clone size, a significant proportion of clones remained of smaller size (almost 75%) even at 24 weeks after induction. These clones could be labelled with BrdU in label retaining experiments showing their quiescence. Upon TPA stimulation, these clones could proliferate and grow in size.
Our results support the idea that small clones that are not attached to HFs are quiescent and intrinsically different from clones attached to HF. In conclusions, our results support a dual behaviour of epidermal progenitors in the skin depending on their proximity of hair follicles.

**Intractable pruritus as a manifestation of intraductal papillary mucinous neoplasm of pancreas**

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Chronic pruritus is defined as itch lasting for equal to or greater than six weeks. Patients with normal-appearing skin and pruritus are thought to have systemic, neurologic, or psychogenic causes of their itch. Systemic illnesses well known to be associated with pruritus include chronic renal failure, liver disease, thyroid disease, HIV infection, and malignancies\(^1\). Pruritus is a frequent complaint in patients with cancer. It may be an indicator of increased risk of undetected hematologic and bile duct malignancies. However, premalignant cysts of pancreas have rarely been reported in association with chronic pruritic. We present a 72-years-old female with intractable generalised pruritus for three months without significant cutaneous dermatoses. She reported 15 kg weight loss for the last 2 years. Pruritus was not responsive to the general treatments and was affecting her quality of life dramatically. Meticulous baseline investigations for paraneoplastic pruritus failed to reveal any abnormality. She was referred to Oncologist and abdominal MRI revealed a mucinous cystic neoplasm of the pancreas involving head and uncinated process. Whipple’s procedure was performed as a treatment and histopathology confirmed a low-grade intraductal papillary mucinous neoplasm (IPMN) of branch duct type with no invasion. Patient’s pruritus resolved shortly following the procedure. Thorough investigations and close monitoring of patients with intractable pruritus is paramount in early diagnosis of cancerous and precancerous lesions and their treatment.


**Methotrexate induced necrosis in chronic plaque psoriasis**

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Methotrexate (MTX) is a chemotherapeutic drug that acts primarily by inhibiting thymidylate synthase and the folic acid cycle, resulting in impaired nucleic acid synthesis leading to cell death. It is widely used in the treatment of proliferative disorders ranging from neoplasms to psoriasis. Methotrexate is mainly excreted in unchanged form by the kidney. Impaired kidney function delays methotrexate clearance and may lead to serious toxicity. Methotrexate toxicity manifests itself in several forms including hepatotoxicity, pulmonary toxicity, acute renal failure, stomatitis, ulceration/erosion of the gastrointestinal tract, and pancytopenia. Ulceration of psoriatic plaques is a rare complication of methotrexate treatment, which may be the only clinical evidence of toxicity. It has been hypothesised that painful erosion of psoriatic plaques represents
an early cutaneous sign of toxicity. We report an 87-years-old male presented with infected necrotic ulcers of both legs following MTX use for chronic plaque psoriasis. These started two weeks after dose was increased from 7.5mg to 15mg due to the poor response. He had a background of type2 diabetes, hypertension and chronic kidney disease. MTX was ceased and he was treated with Cephalixin, Mupirocin, condy’s soaks and emollients. Ulcers healed significantly within two weeks. It is important that clinicians be aware of skin ulceration as an early sign of MTX toxicity and consider renal function tests before commencing or increasing MTX dose.


Pregnancy-associated erythema annulare centrifugum

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Erythema annulare centrifugum (EAC) has been considered to be related to many factors such as immunological disorders, infections and malignancies. Pregnancy-associated EAC is extremely rare.

A 34-year-old woman presented with asymptomatic skin lesions on her legs in the 36th week (36w) of her first pregnancy. Her medical history was unremarkable. The skin lesions had initially appeared in 10w, and then spontaneously regressed within two months. Subsequently, they reappeared in 28w and gradually enlarged and increased in number. Clinical examination showed multiple annular erythematous plaques with slightly raised borders and trailing scales at the inner border of rims. Histopathology showed mild hyperkeratosis, spongiosis in the epidermis and partially demarcated perivascular lymphocytic infiltration. She was diagnosed as EAC. All the laboratory test results including antinuclear antibodies, anti-SS-A and -SS-B antibodies and rheumatoid factors were normal. Although all the erythematous lesions had been growing until the delivery, they started to disappear within a few days after the delivery and completely resolved within one month without any treatment. No recurrence was observed during six months of follow-up.

Only six cases of pregnancy-associated EAC have been reported in the literature. In all cases including ours, EAC occurred during the first pregnancy, and spontaneously disappeared during the late pregnancy or the early postpartum period. Thus, there are some hypotheses that the changes of hormone levels are etiological. The fact that the initial lesions, which appeared in the early pregnancy, spontaneously disappeared in our case may suggest that gonadotropin plays an important role in EAC.
Immunohistochemical localization of pituitary adenylate cyclase-activating polypeptide in murine and human eccrine sweat glands

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Pituitary adenylate cyclase-activating polypeptide (PACAP) exhibits pleiotropic functions in the central nervous system, including neurotransmission, neuroprotection, and vasodilatation. In the skin, PACAP and its receptors were reported to be important mediators of cutaneous vasoregulation and neurogenic inflammation, moreover some studies suggested a role for PACAP in the several exocrine glands; however, their involvement in eccrine sweat secretion have not been examined.

We previously reported that subcutaneous administration of PACAP into the mouse footpad induces significant sweat secretion in a dose-dependent manner. This effect was mediated through PACAP-specific receptor (PAC1R). In addition, the mRNA expression of PACAP and PAC1R in human and murine sweat glands was examined by RT-PCR. Furthermore, we had shown most PAC1R-immunopositivity was observed in these secretory cells.

This study was undertaken to define the precise localization of PACAP in murine and human eccrine sweat glands. By immunohistochemical staining, PACAP-immunoreactivity was observed in eccrine secretory cells and nerve fibres around the eccrine sweat gland. Double-immunostaining with PACAP and neurofilament-200 (NF-200) showed the co-localization in nerve fibres around the mouse and human sweat glands.

Immunohistochmical localization of PACAP and previous functional study collectively suggest that PACAP may play a crucial role in sweat secretion via its action on PAC1R located in eccrine sweat glands. The mechanisms underlying the role of PACAP in sweat secretion may provide new therapeutic options to combat sweating disorders.

Time course observation of thyroid-associated autoantibody and hormone and profiles in patients receiving nivolumab for metastatic melanoma

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Nivolumab, an anti-programmed death-1 specific monoclonal antibody, exhibits antitumor effect by enhancing host immunoreactivity against tumors and has been known to induce autoimmune adverse events including thyroid dysfunction. To better characterize sequential changes in thyroid-associated hormone and autoantibody profiles, we conducted time course analysis in 4 cases of metastatic melanoma patients treated with nivolumab.

One out of 4 cases had serological evidence of positive antithyroglobulins (anti-Tg) and antithyroid peroxidase antibodies (anti-TPO) with normal free T3, free T4 and thyroid-stimulating hormone levels prior to nivolumab exposure. Intriguingly, increase in anti-Tg and -TPO antibody levels accompanied by rise in free T3 and T4 was detected in all cases 3-7 weeks after the first nivolumab administration. Importantly, some linearity between pretreatment anti-Tg and -TPO levels and the extent of posttreatment thyroid dysfunction was detected. Of note, the case with the highest initial antibody levels demonstrated remarkably elevated anti-Tg and -TPO antibody levels within 3weeks and developed Hashimoto’s disease with eyelid edema and severe general fatigue 10 weeks after the treatment. In other three cases without
clinical signs of hypothyroidism, the rapidity and the peak of anti-thyroid autoantibody production, which remained within normal limits, correlated with pretreatment autoantibody levels.

Further accumulation of the cases is necessary however, these findings suggested that thyroid autoantibody levels before the initiation of nivolumab treatment might predict a potential risk for developing thyroid dysfunction represented by painless thyroiditis syndrome and Hashimoto’s disease. Thus, the evaluation prior to nivolumab administration would enable better management of thyroid-related adverse events.

Pyoderma Gangrenosum, Acne, and Suppurative Hidradenitis (PASH) Syndrome Treated with Granulocyte and Monocyte Adsorption Apheresis

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Pyoderma gangrenosum, acne, and suppurative hidradenitis (PASH) syndrome is described as an autoinflammatory disorder, similar to PAPA syndrome, but without joint involvement. We present a case of PASH syndrome that was treated with granulocyte and monocyte adsorption apheresis (GMA) therapy followed by adalimumab. An 18-year-old man was referred to our department with a 2-year history of fever, severe facial pustules and papules, repeated ulcer formation, and scars, accompanied by pain on the neck and occipital region. Suppurative hidradenitis had recurred since he was 10 years of age. The patient complained of pain in the neck and occipital region, and many scars were observed on the chest, back, and thighs. Laboratory data showed an increased leukocyte count and an elevated C-reactive protein (CRP) level (10.19 mg/dL). Prednisolone (30 mg/day), cyclosporine, dapsone, and minocycline were not effective. The patient received GMA sessions weekly for 10 consecutive weeks. After 2 sessions, the ulcers, the low-grade fever and pain disappeared, and the number of pustules, swelling, and erythema on the face and neck were remarkably reduced. The CRP level decreased to 0.25 mg/dL after the last session. However, pustule formation did not completely disappear. Therefore, adalimumab administration was initiated 1.5 months after GMA. Symptoms improved 2 weeks after the first adalimumab injection, but pustule formation continued despite 14 injections. This is the first report to show that GMA is effective for treating PASH syndrome. We clearly demonstrate that GMA was effective for treating a case of PASH syndrome, though complete remission was not obtained.

A case of anal gland carcinoma in situ with Pagetoid spread showing no macroscopic abnormality in the rectal and anal mucosa

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We report the case of an anal gland carcinoma in situ with Pagetoid spread. An 81-year-old male presented to our clinic with the complaint of anal pain. Physical examination revealed widespread erythema in the perianal skin. A histological examination of the skin lesion revealed Pagetoid spread of large round atypical cells with clear cytoplasm. Immunohistochemical examination revealed CK20 (+) and GCDFP15 (−) tumor cells. A Pagetoid spread of colorectal or anal carcinoma was suspected. However, no abnormality was detected on gastrointestinal examination, including palpation, anoscopy, and colonoscopy. Therefore, an anal mucosal biopsy was performed to examine the subclinical mucosal lesion. In the biopsy specimen, a Pagetoid spread of CK20 (+) and GCDFP15 (−) tumor cells was observed throughout from the anal side to the oral side. The tumor cells proliferated predominantly around the anal
Based on these findings, we made a diagnosis of Pagetoid spread of the anal gland carcinoma. The patient underwent radical resection of the skin lesion and abdominoperineal resection. This case strongly suggests that an anal mucosal biopsy should be performed in cases with perianal CK20 (+) Paget’s disease without macroscopic abnormalities in the lower part of gastrointestinal tract.

Differentiation of murine dermal papilla cells into myogenic lineage

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Background/Objective: Duchenne muscular dystrophy (DMD) is caused by the absence of dystrophin and is potentially amenable to cell-based therapies. Dermal Papilla Cells (DPC) are highly plastic, multipotent cells reprogrammable to bone, cartilage, fat, hematopoietic and myogenic differentiation. We sought to evaluate whether DPC are suitable and effective in cell-based therapies for DMD.

Design/Methods: Mouse dermal papillae were microdissected from hair follicles of the whisker pad and tissue cultured. DPC cells from the outgrowths were co-cultured with primary mouse lamin-null myoblasts, H-2K myoblasts or normal and dystrophic human myoblasts. Contributions of DPC into myotubes were identified and measured, using lamin A/C with DAPI counterstaining as markers of myonuclear origin. DPC myogenic differentiation was detected using murine-specific PCR assays of the muscle marker “myogenin”. Effects of three bioactives: Galectin-1, purmorphamine and Shh, on differentiation were investigated.

Results/Discussion: DPC can undergo myogenic differentiation in co-culture with myoblasts. In human co-cultures, DPC-derived murine myonuclei were detected inside myotubes. Murine-specific PCR assays showed up-regulation of DPC-derived myogenin, suggesting that they underwent myogenic differentiation. None of the treatments increased myogenin expression in DPC; but, triggering Shh signaling produced a dose-dependent pattern whereby lower levels of signaling promoted myogenic differentiation while higher levels inhibited it. Activating Shh signaling upstream of Smo via purmorphamine, induced a biphasic differentiative response; however the application of Shh hindered the differentiation of both cell types. Thus, murine DPC can undergo myogenic differentiation in vitro. We aim to improve their differentiation efficacy to make them suitable candidates for therapeutic applications in DMD.

Microbiopsy biomarker profiling in a case of melanoma resembling a pigmented basal cell carcinoma

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We present a case of an 84 year old man, with generally sun-damaged skin and previous non-melanoma skin cancers, who presents with a 8 mm pigmented lesion on his mid back. Dermoscopy of the lesion showed grey ovoid nests, spoke-wheel pattern and some atypical pigment network on the peripheries. Arborizing telangiectasia was absent. These features raised the possibility of either a pigmented BCC or a melanoma.

Microbiopsies, a minimally-invasive, painless method of sampling the epidermis, were performed on the lesion, followed by a shave biopsy. Quantitative PCR was performed on the patient’s microbiopsy samples as well as on a histopathologically-confirmed pigmented BCC for comparison, looking for melanoma markers Tyr, CMIP and LINC00518 and a BCC biomarker, Gli1.
The results showed that Gli1 was significantly overexpressed in pigmented BCC sample (24-fold) compared to our patient’s. LINC00518 was upregulated in our patient’s sample (47-fold) which is consistent with a previous report. We did not observe a difference in the expression of CMIP between these samples despite this transcript being a published melanoma biomarker.

The histological diagnosis showed a lentiginous melanoma arising from moderately to severely sun-damaged skin, Breslow thickness 0.2mm, Clark level 2. There was no dermal mitotic activity. Tumour-infiltrating lymphocytes were non-brisk. Regression 1.2 mm was present. Focal ulceration was absent.

We conclude that there was agreement between our microbiopsy RNA profiling and histological diagnosis and that microbiopsies can be a useful diagnostic tool in clinically ambiguous lesions.

Clinical, Dermatoscopic and Histological features of trial melanomas in the High Risk Clinic (HRC) Study: Melanoma Institute, Australia (MIA).

Phoebe Star, Pascale Guitera

Background: The HRC study commenced at MIA in Sydney in 2011 as part of a multicentre prospective cohort study examining the impact of digital dermatoscopic surveillance and total body photography on melanoma diagnosis in extreme risk individuals.1

Objectives: (1) To examine clinical, dermatoscopic and histological features of melanomas diagnosed as part of the HRC study at MIA (2) To assess mode of diagnosis and benign to malignant excision ratio for each of the patients diagnosed with melanoma.

Methods: Clinical data, dermoscopy and histopathology of melanomas diagnosed from June 2011 to May 2016 in the HRC study were analysed retrospectively.

Results: 31 melanomas were detected in 21 of the 84 patients during a median follow-up of 2.7 years. 11 were in situ, 11 were ≤ 1mm invasive melanomas, 1 was >1mm and there was 1 local recurrence. 7 had overlapping dysplastic histology where melanoma could not be ruled out. 24 dermatoscopic images were available. 8 melanomas exhibited no specific criteria according to the Menzies’s Method but displayed atypical network/vessels, milky red colour or change on monitoring. 10 melanomas fulfilled one positive feature for melanoma and 6 melanomas had two or more positive features of melanoma. Benign to malignant excision ratio and mode of diagnosis will be discussed.

Conclusions: An examination of these melanomas provides an opportunity to examine clinically subtle dermatoscopic patterns of early melanoma, as well as mode of detection and rates of excision in individuals under intense skin surveillance.


High-risk squamous cell carcinoma masquerading as dense inflammation on Mohs frozen sections

Thomas J Stewart, Liang Joo Leow

Limitations of frozen sections in detecting moderately- to poorly-differentiated squamous cell carcinoma (SCC) may result in high-risk tumour masquerading as dense inflammation during Mohs micrographic surgery (MMS). While standard histopathological staining of paraffin sections in such situations provides superior diagnostic accuracy,1 this too is not infallible. We present a case of an 82-year-old female who required pan-cytokeratin (immunohistological) staining of frozen sections to diagnose correctly poorly-differentiated SCC manifesting as dense inflammation and scarring on standard staining. There is evidence
for rapid immunostaining at the time of MMS. Notwithstanding cost and practicality issues, the availability of rapid immunostaining may relinquish the need for such tissue to be submitted for permanent section.


Case Presentation: A rare case of bilateral parotid squamous cell carcinoma in a 62 year-old male

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Squamous cell carcinoma (SCC) of the parotid gland is a rare, high-grade malignant tumour, with high mortality. Bilateral presentation of this condition points to a likely metastatic process, commonly from a cutaneous, mucousal or lung epithelial primary tumour that may also be locally invasive and aggressive.

IW presented to his GP after a sudden onset over 2 weeks of gradually enlargening painless parotid swellings. Fine-needle aspirate showed bilateral parotid squamous cell carcinoma on histopathology.

In this case, the primary lesion was never identified either on full skin examination, ENT investigation, or high-resolution CT PET scanning. IW had a history of basal cell carcinoma on his back 3 years prior, was a non-smoker, and was not actinopathic.

Radical bilateral parotidectomy plus right neck dissection was attempted as curative treatment, with consideration of adjuvunct radiotherapy.

This case interestingly points out that SCC may evolve into metastatic disease with spontaneous resolution of the primary lesion, or that whole-body high-resolution CT scanning may fail to diagnose primary disease in such cases.

I intend to perform a literature search and discuss this unusual and rare case.

The melanoma-enriched microRNA miR-4731 regulates genes involved in cell cycle and the melanosome

Mitchell S Stark, Lisa N Tom, Glen M Boyle, Vanessa F Bonazzi, Adrian C Herington, Pamela M Pollock,

We previously identified miR-4731-5p (miR-4731) as a melanoma-enriched microRNA following comparison of melanoma with other cell lines from solid malignancies. Additionally, miR-4731 has been found in serum from melanoma patients and expressed less abundantly in metastatic melanomas from stage IV patients relative to stage III patients. As miR-4731 has no known function, we used biotin-labelled miRNA duplex pull-down to identify binding targets of miR-4731 in three melanoma cell lines. Using the miRanda miRNA binding algorithm, all pulled-down transcripts common to the three cell lines (n=1092) were predicted to be targets of miR-4731 and gene-set enrichment analysis of these (via STRING v9.1) highlighted significantly associated genes related to the ‘cell cycle’ and ‘melanosome’ pathways. Following miR-4731 overexpression, a selection (n=81) of pull-down transcripts underwent validation using a custom qRT-PCR array. These data revealed that miR-4731 regulates multiple genes associated with the cell cycle (e.g. CCNA2, ORC5L, and PCNA) and melanosome (e.g. RAB7A, CTSD, and GNA13). Furthermore, members of the synovial sarcoma X breakpoint family (SSX) melanoma growth promoters were also down-regulated (e.g. SSX2, SSX4, and SSX4B) as result of miR-4731 overexpression. We therefore speculate that loss of miR-4731 expression supports melanoma growth by, in part; reducing its regulatory control of SSX expression levels together with members of the cell cycle pathway, which warrants further investigation.
In the history of modern dermatology, the name of Ferdinand von Hebra (1816-1880) stands conspicuous in the pantheon of historical greats. Born into relative obscurity 200 years ago in the Moravian city of Brünn (Brno), Hebra rose to become one of the most respected Professors of Viennese medicine, and secured himself lasting fame through originally establishing the New Vienna School of Dermatology. Helping to more fully dispel Hippocratic humoralism from conceptions of skin disease, Hebra was able to recognize the importance of external factors in the pathogenesis of a variety of cutaneous eruptions, and hence appreciated the value of topical therapy in a way not realized by his predecessors. He classified skin diseases on new and objective lines, and unified various skin disorders in ways that could be easily understood and verified by others. Apart from naming ‘erythema multiforme’ (sometimes called ‘Hebra’s disease’), and more fully describing a number of other well-defined skin conditions (scabies, ichthyosis planus, discoid lupus, lichen scrofulosorum, impetigo herpetiformis, and rhinoscleroma), Hebra was widely acknowledged for his systematic lectures and demonstrations at the Vienna General Hospital, where he occupied the first ever chair of dermatology. These lectures provided the impetus for a number of Hebra’s distinguished students to follow in his wake, and set on firm foundations the emerging art of dermatology as a distinct specialty. Illustrated with wonderful images from his grand Atlas of Skin Diseases (1856-1876), this poster pays timely homage to Hebra on the bicentenary of his birth.
Dermatofibrosarcoma protuberans masquerading as CBCL

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A 27-year-old otherwise healthy woman was presented at Dermatology Department in July 2015 with a cutaneous lesion of the abdomen. The lesion first developed six months earlier, and gradually enlarged. Physical examination revealed a dark reddish, firm node, 30 mm in diameter on lower abdomen. Its surface was smooth without atrophic or ulcerative parts. Axillary and inguinal lymph nodes were not palpable. We suspected cutaneous B-cell lymphoma (CBCL) on the basis of morphology. A biopsy was performed and histopathological findings demonstrated that the dermis and subcutaneous tissue are replaced by densely packed tumor cells. These cells were spindle-shaped with a little cytoplasm, arranged in a storiform pattern. The majority of them were positive on staining with the antibody CD34 and vimentin, and negative on S-100 protein. 5% of nuclei were Ki67 antigen positive in center of the tumor whereas 10% in the margin. Based on these findings, we made a diagnose of dermatofibrosarcoma protuberans (DFSP). Magnetic resonance imaging (MRI) showed a well-demarcated mass, 28mm × 15mm in diameter, through skin surface to subcutaneous tissue. Wide local excision was conducted including a 2 cm safety margin.

Evaluation of the anti-tumor effect of HVJ-E on melanoma

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Background: As well-known, HVJ-E, the inactivated Hemagglutinating Virus of Japan (HVJ-E) was always used as a pseudovirus for gene and drug delivery, the vector can deliver siRNA, DNA, proteins and anti-cancer drugs to cells in vitro and in vivo. However, recently, HVJ-E is reported to have a strong anti-tumor effect against various tumors.

Aim: In this study, we aimed to confirm the anti-melanoma effect in mice, and further investigate the exact mechanism about it.

Materials and Methods: In vivo, 6 weeks old male C57BL/6J mice were intradermal inoculated with 500,000 mouse melanoma F10 cells, 10 days after tumor inoculation, 1,500 HAU of HVJ-E was injected into tumor. Then the tissue of mice melanoma was analyzed by HE staining and tunnel staining. In Vitro, cultured F10 melanoma cells were treated with HVJ-E, then cell morphology alterations and cell death was analyzed by microscopic observation and western blot analyses.

Results: we found 3 days after HVJ-E injection, the black melanoma tissue turned white, a dramatic anti-tumor effect was observed in mice melanoma. Furthermore, cell fusion and apoptotic cell death were observed in early phase after HVJ-E treatment, both in vivo and in vitro.

Conclusion: Our results suggest that HVJ-E treatment has a potent effect on melanoma via cell fusion and apoptotic cell death.
A case-control study of the associations of PGC1β genotype with melanoma, skin reflectance, and naevus count

Xuan Ling, Hilary Yong, Annette Pflugfelder, David Duffy, Kasturee Jagirdar, Katie Lee, Claus Garbe, H. Peter Soyer, Richard Sturm

As sun exposure and high melanocytic naevus count are two of the most important risk factors contributing to melanoma, we are exploring associations between germline variant alleles in candidate genes involved in tanning capacity, naevus count, melanogenesis and melanoma risk. The PPARC1B gene encoding the PGC1β protein has both coding and noncoding variants, with two intronic SNPs previously associated with increased tanning capacity (rs32579) and decreased naevus count (rs251468). We have performed a genetic association study of these phenotypes in two Case-control populations, one from Australia drawn from the Brisbane Nevus Morphology Study (BNMS) with over 1000 participants, and the second from Germany recruited at the University Hospital Tubingen including 761 volunteers. High throughput SNP genotyping using the Illumina HumanCoreExome-24 Chip, TaqMan assay and Sanger sequencing was performed. We observed that the PGC1β variant at rs45520937 (R226Q) was associated with increased pigmentation on the inner and outer forearm (p=0.039 and p=0.015 respectively), based on Sanger Sequencing of PGC1β exon 5 in 184 patients from the BNMS. Linkage disequilibrium was also detected between rs7732671 (A203P) and rs17572019 (V204I). Moreover, the presence of a minor allele for a PGC1β variant, rs7732671 (A203P), predicted decreased survival probability in stage IV melanomas in the German samples. These findings support the association between melanoma progression and PGC1β germline variants. Further analysis with all PGC1β variants and other candidate genes is continuing including additional BNMS patients to enable a better understanding of the genetic and environmental influences in melanoma etiology.

Are we spinning yarns? Report from the Determining Effects of Superfine Sheep-wool in INFantile Eczema (DESSINE) study group on the effects of wool in atopic dermatitis

Michaela Zallmann, Pete K Smith, Mimi L Tang, Jenny Cahill, Lynda Spelman, Constance H Katelaris, Katie J Allen, John Su

Wool worn against the skin is frequently perceived as prickly and pruritogenic. Similarly, in the medical community, wool has a common reputation as a cutaneous irritant. Avoidance of wool garments, in favour of cotton, has been advocated for patients with atopic dermatitis (AD) since the 1930’s. Moreover, many patients also indiscriminately equate itch with allergy and self-identify as being allergic to wool. These beliefs have persisted despite new insights into itch transmission and the fibre properties responsible for pruritoperceptor activation.

We present the findings of a literature review of published articles from the last 100 years obtained from a search of MEDLINE and Google Scholar using key search terms, examining the evidence for the potential of wool to: i) exacerbate AD, ii) cause irritant contact dermatitis and iii) cause allergic contact dermatitis. We also review the capacity for modern woollen garments to activate c-fibres for itch transmission.

There is a lack of current evidence to indicate that the wool fibre is an allergen or that it has a role in allergic exacerbations of AD, contact urticaria and allergic contact dermatitis. Cutaneous irritation reported for wool garments in previous years can be attributed to high fibre diameters. Superfine and ultrafine Merino wool with finer fibre diameters do not activate sufficient c-fibres responsible for itch transmission and appear well tolerated and potentially beneficial in AD management.
Evolution of 164 melanocytic naevi under metastatic melanoma systemic therapies

Cathy Y Zhao, Shelley Ji Eun JE Hwang, Deepal Wakade, Giuliana Carlos, Pablo Fernandez-Penas

Introduction: Treatment of metastatic melanoma with systemic therapies is associated with cutaneous side effects, including morphological changes in pre-existing naevi.1-3 We sought to review naevi evolution pattern amongst four systemic melanoma therapies: anti-programmed cell death protein 1 (anti-PD 1) therapies including pembrolizumab and nivolumab, anti-PD 1 combined with ipilimumab therapy, selective BRAF-inhibitors including dabrafenib, vemurafenib and encorafenib, and BRAF-inhibitor combined with trametinib therapy.

Method: Patients seen at Westmead Hospital onco-dermatology clinic between February 2013-February 2016 that had their naevi dermatoscopically monitored using Fotofinder during their routine follow-up visits were included in the study. Changes in naevi colour were examined using the Fotofinder's mole analyser.

Results: Forty patients with a total of 164 naevi were monitored. All except 2 patients had their serial photos taken within 1 year of their baseline. The anti-PD 1 group had 11 patients and 46 naevi; 47.8% of the naevi lightened, 43.5% unchanged and 8.7% darkened. The anti-PD 1 combined with ipilimumab group had 10 patients and 38 naevi; 65.8% of the naevi lightened, 21.1% unchanged and 13.2% darkened. The BRAF-inhibitor group had 11 patients and 39 naevi; 41.0% of the naevi lightened, 23.1% unchanged and 35.9% darkened; The BRAF-inhibitor combined with trametinib group had 7 patients and 41 naevi; 61.0% of the naevi lightened, 36.6% were unchanged 2.4% darkened.

Conclusion: The BRAF-inhibitors appear most likely to cause darkening of naevi while the BRAF-inhibitor combined with trametinib group and the anti-PD 1 combined with ipilimumab group appear most likely to cause lightening of naevi.

A multi-center validation study of atopic dermatitis scores in patients of dark skin

Cathy Y Zhao, Evelyn Hao, Daniel Oh, Benjamin S Daniel, Linda K Martin, John C Su, Michelle A Rodrigues, Murrell F Dedee

Background: Atopic dermatitis (AD) scores, including the recommended Eczema Area Severeity Index (EASI), appeared unreliable in dark skinned patients in a previous study.1

Aim: To compare the inter-rater and intra-rater reliability of various AD scores in real-life dark skin patients from two centres in Australia.

Method: 25 AD patients each attended a one-day scoring exercises based in either Sydney or Melbourne. Each patient was scored by 5 dermatology doctors using the EASI2, objective-Scoring Atopic Dermatitis score (oSCORAD), Physician Global Assessment (PGA) and a grey-scale composed of four shades of grey. A Mexameter was used for their baseline melanin indices. Ten patients were re-scored for intra-rater reliability testing.

Results: There were 14 dark skinned patients (melanin index>200) and 11 light skinned patients (melanin index≤200). The inter-rater ICCs of each score were: EASI 0.83 (95%CI 0.66-0.94) in light skinned patients and 0.77 (95%CI 0.60-0.91) in dark skinned patients; oSCORAD 0.68 (95% CI 0.44-0.88) in light skinned patients and 0.74 (95%CI 0.54-0.89) in dark skinned patients; PGA 0.80 (95%CI 0.62-0.93) in light skinned patients and 0.70 (95%CI 0.49-0.87) in dark skinned patients; the grey-scale had an inter-rater ICC of 0.64 (95%CI 0.40-0.84) for dark skin patients. Intra-rater ICCs of all scores were excellent in all skin types. Separate erythema component calculations showed that erythema did not contribute to the scores' variability. The SCORAD's erythema components had higher inter-rater variations than the EASI’s.

Conclusions: The EASI score had excellent inter-rater reliability for all skin colors, while the SCORAD had good reliability for all skin colors.
Angiomatoid fibrous histiocytoma of the scalp mimicking cutaneous angiosarcoma: Case report

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Angiomatoid fibrous histiocytoma (AFH) is a rare soft tissue tumor that has been categorized as a tumor of uncertain differentiation and intermediate malignancy. In the previous literature, it has not been emphasized that the histological and immunohistochemical features of AFH can mimic those of cutaneous angiosarcoma (AS). Nonetheless, AFH may conceivably be misdiagnosed as AS in medical practice. This potential diagnostic pitfall may be especially likely when the diagnosis is based on small biopsy specimens of head lesions. We report a case of AFH of the scalp with a reticular pattern in a 29-year-old Japanese man, whose biopsy specimen of the lesion was histologically diagnosed as AS. The combination of the following three histopathological features was misleading in our case: nuclear pleomorphism, pseudovascular reticular space, and the CD31 immunoreactivity of numerous intervening histiocytes. In conclusion, we should consider the differential diagnosis between AS and AFH in terms of these histopathological features of soft tissue tumors with vascular and angiomatous differentiation.