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The US FDA 'Black Box' Warning for Topical Calcineurin Inhibitors

An Ongoing Controversy

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Abstract

Atopic dermatitis is a chronic inflammatory skin disease characterized by recurrent intense pruritus and a distinctive distribution of skin lesions. The topical calcineurin inhibitors tacrolimus and pimecrolimus were approved in the USA, as an ointment and a cream, respectively, for the treatment of atopic dermatitis in 2000 and 2001, respectively. In 2005, the Pediatric Advisory Committee of the US FDA implemented a 'black box' warning for tacrolimus ointment and pimecrolimus cream due to the lack of long-term safety data and the potential risk of the development of malignancies. This article focuses on the safety aspects of these agents by discussing the findings from preclinical and clinical studies and postmarketing reports with regard to malignancies occurring after the use of tacrolimus ointment and pimecrolimus cream.

Atopic dermatitis, also described as atopic eczema or just eczema, is defined clinically as an inflammatory, chronically relapsing, pruritic skin disease with a typical age-related distribution of lesions.[1-6] The worldwide prevalence of atopic dermatitis has increased during recent decades, especially in industrialized countries, with a current prevalence in children of up to 20%.[7-13] The impact of atopic dermatitis on patients and their families is comparable to other important noncommunicable diseases and may also cause substantial psychiatric co-morbidity.[13]

To prepare this article, we searched the MEDLINE database for the terms 'atopic eczema', 'atopic dermatitis', 'pimecrolimus cream', 'tacrolimus ointment', 'topical calcineurin inhibitor', 'cancer', 'neoplasm' and 'lymphoma' to identify published papers available in the English language. Wherever appropriate, references in book chapters or on websites were added. All papers and book chapters compiled until April 2007 were checked for suitability for this article by all authors.

1. Topical Treatment of Atopic Dermatitis

The management of atopic dermatitis is complex and compromises a variety of therapeutic and preventive measures. In the acute phase, anti-inflammatory management is mandatory.

1.1 Topical Corticosteroids

Topical corticosteroids have been the standard treatment for atopic dermatitis for half a century.[14] However, they carry the risk of local adverse effects, such as skin atrophy, and other dermatological conditions, such as rosacea-like dermatitis.[15] Systemic complications associated with the long-term use of

mid- to high-potency topical corticosteroids include growth retardation and abnormal adrenocortical function.[16,17] In addition, a reduction in the responsiveness of lesions to the application of topical corticosteroids ('tachyphylaxis') may develop,[18] making other therapeutic approaches advisable.

1.2 Topical Calcineurin Inhibitors

The topical calcineurin inhibitors tacrolimus and pimecrolimus are potent anti-inflammatory substances without the adverse effects associated with corticosteroids.

Tacrolimus ointment was launched in December 2000. The 0.03% and 0.1% concentrations (adults) and the 0.03% concentration (children aged 2–15 years) are indicated and labelled in the US as second-line therapy for the short-term and non-continuous long-term treatment of moderate to severe atopic dermatitis in non-immunocompromised adults and children who have not responded adequately to other topical treatments or when those treatments are not advisable.[19]

In December 2001, pimecrolimus 1% cream received approval by the US FDA. The cream is indicated as second-line therapy for the short-term and non-continuous long-term treatment of mild to moderate atopic dermatitis in non-immunocompromised adults and children 2 years of age or older, who do not respond adequately to other topical treatments, or when those treatments are not advisable.[20]

The two drugs have similar chemical structures and a nearly identical mechanism of action: they inhibit T helper cell (T_h)1- and T_h2-derived cytokine production as well as inhibiting the release of mediators from mast cells and basophils by binding to FK506 binding protein-12. This complex selectively inhibits calcineurin, which is required to activate the transcription factor of activated T cells (FAT) in T lymphocytes.[21-23]

The most common treatment-related adverse effect of topical calcineurin inhibitors is a sensation of burning at the application site, which is typically mild to moderate in severity, decreases after the first few days of treatment and rarely leads to treatment discontinuation.[24,25] In contrast to topical corticosteroids, topical calcineurin inhibitors do not seem to be atrophogenic.[26-28]

2. The US FDA Perspective on Topical Calcineurin Inhibitors

In January 2005, the FDA raised concerns about the use of topical pimecrolimus and tacrolimus in atopic dermatitis, suggesting that there might be a potential risk of development of malignancy in terms of lymphoma and skin cancer.[29] On 15 February 2005, the Pediatric Advisory Committee of the FDA recommended a 'black box' warning, which represents the highest level of five possible warning categories found in the package insert, for the topical use of pimecrolimus and tacrolimus because of a lack of data on long-term safety and a potential risk of cancer (see figure 1).[30]

On 10 March 2005, the FDA issued a Public Health Advisory informing healthcare professionals of their safety concerns associated with the use of these drugs.[31] As described by Fonacier and coworkers,[30] the following concerns urged the FDA to implement the 'black box' warning:

1. the increasing use of topical calcineurin inhibitors as first-line therapy and off-label in atopic dermatitis;
2. heavy direct-to-consumer advertising of topical calcineurin inhibitors to patients with atopic dermatitis and parents of children with atopic dermatitis, which might be related to their increasing use;
3. postmarketing reports of malignancy in children and adults who had used topical calcineurin inhibitors;
4. a postmarketing nonhuman primate study with an oral formulation of pimecrolimus, which demon-

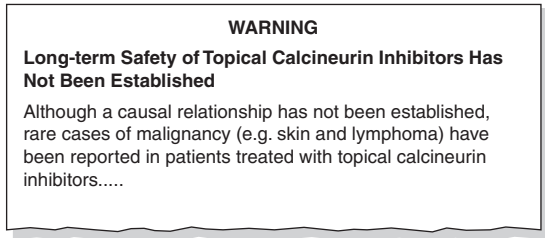

Fig. 1. Topical calcineurin inhibitors: US FDA 'black box' warning.

strated an occurrence of lymphoma in monkeys exposed to the lowest dose (equivalent to 30 times the maximum recommended human dose [MRHD]);
5. the uncertainty about the potential risk of carcinogenicity from topical calcineurin inhibitors, which will remain unknown for years, and concerns about the adequate communication of this to the patient and prescriber to assist them in the proper use of these products;
6. the possible photo-cocarcinogenicity in rodent studies.

This paper will focus on the issues raised in points 3–6.

3. Data on Malignancy with Topical Calcineurin Inhibitors

3.1 Human Keratinocyte Studies

Topical calcineurin inhibitors are thought to avoid systemic immune suppression and, therefore, not have the same risk of skin cancer generation as ciclosporin.

Yarosh et al.[32] examined the effect of the calcineurin inhibitors ciclosporin and ascomycin on DNA repair in normal human keratinocytes after ultraviolet (UV) B irradiation.

They found that the ciclosporin and ascomycin inhibited the removal of cyclobutane pyrimidine dimers, and that they also inhibited UVB-induced apoptosis.[32] Furthermore, UVB irradiation induced nuclear localization of the transcription factor FAT, and this was blocked by ciclosporin as well as ascomycin.[32] Unfortunately, the ascomycin-related drugs pimecrolimus and tacrolimus were not involved directly in the studies conducted by Yarosh et al.,[32] limiting the applicability of these findings to these topical calcineurin inhibitors.

3.2 Carcinogenicity and Photo-Cocarcinogenicity in Rodents

The standard approach to test for photo-cocarcinogenicity of topical substances is the hairless mouse model.[33]

The effect of UVB radiation on hairless mouse skin treated with tacrolimus ointment 0.1% and pimecrolimus cream 1% was investigated by Tran et al.[34,35] Mice were treated once daily, followed by UVB radiation, over a 10-day period. Neither tacrolimus nor pimecrolimus increased DNA damage following UVB radiation. In contrast, mice treated with either of the topical calcineurin inhibitors and UVB radiation showed substantially less epidermal DNA damage than those treated with vehicle only and UVB radiation.

Three 52-week studies assessing the photo-carcinogenic potential of topical tacrolimus and pimecrolimus in hairless mice were conducted based on the standard protocol recommended by the FDA.

In the pimecrolimus study,[36] four groups of hairless albino mice were treated daily, 5 days per week, on the back and sides of the body (an area of approximately 25 cm^2) with pimecrolimus cream 1%, 0.6%, 0.2% or the vehicle cream. A further two groups of mice did not receive any topical application and acted as controls. All mice were exposed to simulated sunlight, including UVB, for 40 weeks. Five groups received doses of 600 Robertson-Berger units (RBU) per week, while one control group received 1200 RBU per week. The mice were followed-up for 12 weeks and the incidence of human papilloma virus (HPV)-induced skin papillomas was monitored. HPV-induced skin papillomas are common in ageing mice, and a minority of them can transform into squamous cell carcinoma (SCC). The tendency is strain-specific. The median times to the appearance of papillomas of >1 mm in diameter in each group are shown in table I.

As shown in table I, topical administration of the vehicle formulation enhanced photo-carcinogenesis. Topical administration of the pimecrolimus formulations at 0.2%, 0.6% and 1% concentrations had no influence on skin tumour development beyond that seen with the vehicle in males. In females, pimecrolimus cream increased the papilloma-free time interval, indicating that the active ingredient pimecrolimus is not photo-carcinogenic.[36]

Table I. Pimecrolimus cream: median time to papilloma appearance in the photo-carcinogenicity study in hairless mice (reproduced from Ring et al.,[36] with permission from Wiley-Blackwell Publishing Ltd)

Treatment group	UVR exposure (RBU/wk)	Median time (wk)	
		males	females
Control 1	600	38	35
Control 2	1200	21	20
Vehicle cream	600	25*	27*
0.2% cream	600	27	34†
0.6% cream	600	25	30††
1% cream	600	26	30††

RBU = Robertson-Berger units; **UVR** = ultraviolet radiation; * $p < 0.001$ vehicle vs control 1; † $p < 0.001$ compared with vehicle; †† $p < 0.005$ compared with vehicle.

In the 52-week tacrolimus study,[36] hairless mice were treated with 600 RBU per week UV radiation and tacrolimus ointment 0.03% and 0.1% or vehicle ointment. A further two groups of mice did not receive any topical application and acted as controls. Four groups received 600 RBU per week, while one control group received 1200 RBU per week. At a UV radiation dose of 600 RBU per week, the median time to onset of skin tumour formation was decreased following long-term topical treatment with vehicle compared with UV radiation alone. In females, an increase in the median time to onset of skin tumour formation was shown for the 0.03% and 0.1% formulations compared with vehicle ointment controls. In males, a decrease in the median time to onset of papilloma appearance (2 weeks for 0.03% tacrolimus ointment; 3.5 weeks for 0.1% tacrolimus ointment was seen (table II).

In a further 52-week study of dermal photocarcinogenicity in hairless mice, the median time to onset of skin tumour formation was decreased following long-term topical administration of pimecolimus cream vehicle alone with concurrent exposure to UV radiation (40 weeks of treatment followed by 12 weeks of observation). No additional effect on tumour development beyond the vehicle effect was noted with the addition of the active agent (pimecrolimus) to the vehicle cream.[20]

Interestingly, in this context, neither pimecrolimus nor tacrolimus induced phototoxicity or photosensitization in human dermal safety studies.[20,37]

In a 2-year rat dermal carcinogenicity study using pimecrolimus cream 1%, a statistically significant increase in the incidence of follicular cell adenoma of the thyroid was found in male animals receiving low, mid and high doses compared with male animals receiving vehicle and saline controls.[20] Follicular cell adenoma of the thyroid was noted in this study at the lowest dose of 2 mg/kg/day 0.2% pimecrolimus cream, equivalent to 1.5 × the MRHD based on area under the concentration-time curve (AUC) comparisons.

No increase in the incidence of follicular cell adenoma of the thyroid was noted in an oral carcinogenicity study in male rats up to 10 mg/kg/day (66 ×

Table II. Tacrolimus ointment: median time to papilloma appearance in the photo-carcinogenicity study in hairless mice (reproduced from Ring et al.,[36] with permission from Wiley-Blackwell Publishing Ltd)

Treatment group	UVR exposure (RBU/wk)	Median time (wk)	
		males	females
Control 1	600	44.5	42
Control 2	1200	28	28.5
Vehicle ointment	600	33.5	36
0.03% ointment	600	31.5	44
0.1% ointment	600	30	42

RBU = Robertson-Berger units; **UVR** = ultraviolet radiation.

MRHD based on AUC comparisons). There was no discernible effect on the development of cutaneous tumours for dermally administered pimecrolimus.[20]

In a mouse epidermal carcinogenicity study[20] using pimecrolimus in an ethanolic solution, no increase in the incidence of neoplasia was observed in the skin and other organs up to the highest dose of 4 mg/kg/day (0.32% pimecrolimus in ethanol), corresponding to 27 × MRHD based on AUC comparisons. However, lymphoproliferative changes (including lymphoma) were noted in a 13-week repeat dose dermal toxicity study[20] conducted in mice using pimecrolimus in an ethanolic solution at a dose of 25 mg/kg/day corresponding to 47 × MRHD, based on AUC comparisons. Interestingly, no lymphoproliferative changes were noted in this study at a dose of 10 mg/kg/day corresponding to 17 × MRHD based on AUC comparisons.

With an increase in dosage (100 mg/kg/day; 179–217 × MRHD based on AUC comparisons), the latency time to lymphoma formation was shortened to 8 weeks after dermal application of pimecrolimus in an ethanolic solution.[20] There was no discernible effect on the development of cutaneous tumours for dermally administered pimecrolimus.

In a mouse oral (gavage) carcinogenicity study, a statistically significant increase in the incidence of lymphoma was noted in male and female animals receiving high doses of pimecrolimus compared with those receiving the vehicle control.[20] Lymphomas were noted at a dosage of 45 mg/kg/day (258–340 × MRHD based on AUC comparisons), but not at a dosage of 15 mg/kg/day (60–133 × MRHD based on AUC comparisons). There was no discernible effect on the development of cutaneous tumours for orally administered pimecrolimus.

In a rat oral (gavage) carcinogenicity study,[20] a statistically significant increase in the incidence of benign thymoma was noted in male rats receiving 5 mg/kg/day pimecrolimus and male and female rats receiving 10 mg/kg/day pimecrolimus compared with animals treated with vehicle control. No drug-related tumours were noted at a dose of 1 mg/kg/day (1.1 × MRHD based on AUC comparisons; male rats) and at a dose of 5 mg/kg/day (21 × MRHD based on AUC comparisons; female rats).[20] There was no discernible effect on development of cutaneous tumours for orally administered pimecrolimus.

Oral carcinogenicity studies with systemically administered tacrolimus have been carried out in male and female rats and mice. In an 80-week mouse study and a 104-week rat study, no relationship between tumour incidence and tacrolimus dosage was found at daily doses up to 3 mg/kg (9 × MRHD based on AUC comparisons) and 5 mg/kg (3 × MRHD based on AUC comparisons), respectively.[19] There was no discernible effect on the development of cutaneous tumours for orally administered tacrolimus.

A 104-week epidermal carcinogenicity study was performed in mice with tacrolimus ointment (0.03–3%), equivalent to tacrolimus doses of 1.1–118 mg/kg/day or 3.3–354 mg/m^2/day.[19] In this study, the incidence of skin tumours was minimal and the topical application of tacrolimus was not associated with skin tumour formation under ambient room lighting. However, a statistically significant elevation in the incidence of pleomorphic lymphoma in high-dose male (25/50) and female (27/50) animals and in the incidence of undifferentiated lymphoma in high-dose female animals (13/50) was noted. Lymphomas were detected in this study at a daily dose of 3.5 mg/kg (0.1% tacrolimus ointment; 26 × MRHD based on AUC comparisons). No drug-related tumours were seen at a daily dose of 1.1 mg/kg (0.03% tacrolimus ointment; 10 × MRHD based on AUC comparisons).[19]

3.2.1 Multistage Mouse Trials of Carcinogenesis

In this model, animals are first treated with the tumour initiating agent 7,12-dimethylbenz[a]anthracene (DMBA) at time 0 and after a 2-week period, followed by biweekly treatment with a tumour promoting agent, such as 12-O-tetradecanoylphorbol-13-acetate (TPA). With this treatment protocol, papillomas (benign lesions) are first visible at 6–8 weeks and carcinomas are initially detected at approximately 30 weeks.[38]

In such a vehicle-controlled, DMBA-initiated and TPA-promoted model, Jiang et al.[39] found that

twice weekly topical applications of 0.1–1.0 μmol/L preparations of tacrolimus ointment in acetone to the skin of CD-1 mice 15 minutes before TPA treatment over a 22-week period markedly inhibited skin tumour (papilloma) formation in a dose-related manner. Skin tumour formation was reduced by up to 80% in mice treated with DMBA + TPA + tacrolimus compared with DMBA + TPA-treated mice. These results indicate that tacrolimus inhibits TPA-induced tumour promotion.

In contrast to the above-mentioned results, Niwa et al.[40] found that daily application of a 0.06 μmol/L formulation of tacrolimus ointment to the skin of CD-1 mice 2 hours after TPA treatment over a 20-week period led to a significant increase in the development of skin tumours between weeks 14 and 20 in some mice treated with DMBA + TPA + tacrolimus compared with DMBA + TPA-treated mice. This significant difference was due to an increase in the number of papillomas developing in each mouse; neither the number of mice affected by skin tumours nor the number of SCCs were significantly different between the two groups. The percentage of tumours classified as SCCs in the DMBA + TPA + tacrolimus group was substantially lower than in the DMBA + TPA group (5.2% vs 13.3%).[40]

Further comparison of the exposure protocols by Jiang and co-workers[39] and Niwa et al.[40] reveals that the first scheme with twice-weekly application of tacrolimus 15 minutes before application of TPA is designed to result in peak local tacrolimus levels at the moment of exposure to the tumour promoter TPA.[39] The inhibition of tumour formation, as observed by Jiang and co-workers,[39] suggests an inhibitory effect of tacrolimus on TPA-mediated neoplasia.

Conversely, the increased tumour formation observed by Niwa et al.,[40] which is associated with lower local pimecrolimus drug levels at the moment of TPA exposure, might be induced by systemic immunosuppression.[41]

3.3 Porcine and Primate Trials

In a 52-week study of minipigs treated with up to 3% tacrolimus ointment on 40% of body surface areas, no dermal carcinogenic effects were reported. The observed blood concentrations of tacrolimus were similar to those in humans receiving treatment with tacrolimus ointment of concentrations up to 3%.[37]

A 39-week oral monkey toxicology study was conducted with pimecrolimus at dosages of 15, 45 and 120 mg/kg/day. A dose-dependent increase in expression of immunosuppressive-related lymphoproliferative disorder (IRLD) associated with lymphocryptovirus, a monkey strain of virus related to the human Epstein Barr virus (EBV), was observed.[20]

IRLD in monkeys mirrors that which has been noted in human transplant recipients after chronic systemic immunosuppressive therapy and post-transplantation lymphoproliferative disease (PTLD). Both IRLD and PTLD can progress to lymphoma, which is dependent on the dose and duration of systemic immunosuppressive therapy. IRLD occurred at the lowest dosage of 15 mg/kg/day for 39 weeks (31 × MRHD of pimecrolimus cream 1% based on AUC comparisons). A partial recovery from IRLD was noted upon cessation of administration of pimecrolimus.[20]

3.4 Limitations of Animal Models

In general, it has to be stated that oral studies with pimecrolimus and tacrolimus do not reflect continuous exposure or have the same metabolic profile as topical dermal application.[36]

Although basic cell biology and the pathogenesis of cancer have similarities between species, animal models do not allow a definitive assessment of the carcinogenic potency of substances in humans. There may be substantial differences in pharmacokinetic response as a result of differences between species.[42]

The skin of humans and mice differs considerably in physiological properties, with mice having more permeable skin than humans.[43] Furthermore,

improvement in skin barrier function and the reduction of UV absorption resulting from the use of topical calcineurin inhibitors in patients with atopic dermatitis is not mimicked in the mouse model.[42]

In addition, prolonged UV exposure eventually causes tumours in all exposed animals, whereas in humans, even Caucasian populations, the rate of sunlight-induced skin cancer is rarely more than a few percent.[33] Substances shown to be carcinogenic in mice, for example retinoic acid, have not been shown to be carcinogenic in humans.[44,45] Surprisingly, there are examples of clinically used vehicles (cream or ointment bases) or drugs in the FDA database that do not absorb UVA, UVB or visible radiation, and yet are capable of enhancing UV-induced carcinogenesis in the mouse model.[46]

In cancer-drug development, numerous agents have shown efficacy in rodent models but have had minimal activity in patients, which has led to reasonable scepticism about the true value of rodent-tumour models in accurately identifying agents that will have important clinical utility.[47]

The biological significance of macroscopically detectable papillomas that are used as endpoints in mouse models is unclear if the nature of these papillomas is not clearly defined. Most tumours depend on the continuous exposure to tumour-promoting chemicals and are reversible after the end of exposure. Some persist without further progression and only a few develop into malignant tumours. The simple counting of tumours without further discrimination of reversibility or persistence may not reflect their biological differences.[42]

The interference of the irritant effects of carcinogenic chemicals may also confound results, as wounding enhances the carcinogenic process in mouse skin.[42,48] In addition, tumour promotion in mouse skin only occurs under specific experimental conditions and predominantly in highly sensitive strains, which may produce test results with uncertain relevance to human skin.[42,49]

In the study by Niwa et al.,[40] on the acceleration of carcinogenesis in mouse skin after topical application of tacrolimus, it is stated that the mice were housed in an air-conditioned room, indicating group accommodation. If this was the case, the housing of the animals in groups may have been a confounding factor, as there may have been scratching between the animals and mechanical irritation of the skin is tumourigenic in mice.[50]

In this 20-week study,[40] the majority of induced tumours were classified as papillomas. Except for HPV-induced tumours, such papillomas are uncommon in humans, and benign. No tacrolimus ointment vehicle control group was assessed, so the active ingredient is unclear. If tacrolimus ointment actually did have tumour-inducing activity, as suggested by the authors, one would expect tumourigenic activity in longer-term trials. However, the 2-year mouse trial failed to show such an effect.[40]

3.5 Further *In Vitro* and *In Vivo* Studies of Topical Calcineurin Inhibitors

In considering the potential direct carcinogenic effects of topical calcineurin inhibitors on keratinocytes, it is possible that they may act as initiators (e.g. mutagens) and/or promoters (e.g. stimulators of proliferation) of neoplasia.[51]

A battery of *in vitro* genotoxicity tests, including the Ames assay, mouse lymphoma L5178Y assay and chromosome aberration test in V79 Chinese hamster cells and an *in vivo* mouse micronucleus test revealed no evidence for a mutagenic or clastogenic potential of pimecrolimus.[20,52] The mouse micronucleus assay found that orally administered pimecrolimus was not associated with any clastogenic potential at doses up to more than 500 times the human therapeutic dose.[20] For tacrolimus, bacterial (*Salmonella sp*. and *Escherichia coli*) or mammalian (Chinese hamster lung-derived cells) *in vitro* assays of mutagenicity, the *in vitro* Chinese hamster ovary cell/hypoxanthine-guanine phosphoribosyltransferase (CHO/HGPRT) assay of mutagenicity or *in vivo* clastogenicity assays conducted in mice failed to show any evidence of genotoxicity.[19] Furthermore, unscheduled DNA synthesis in rodent hepatocytes was not observed.[19]

No assay showed any direct mutagenic or chromosomal-damaging effects attributable to the topical calcineurin inhibitors. Therefore, any carcino-

genic effects attributable to them are much more likely to be the result of indirect activities, e.g. suppression of the host immune system and/or potentiation of the damaging effects of UV radiation.[51]

In contrast to topical corticosteroids, topical calcineurin inhibitors do not affect the maturation of dendritic cells and their antigen-presenting and T-cell stimulating abilities,[53,54] except when they are exposed for extended periods of time to doses of tacrolimus higher than those used in clinical trials.[55]

In human dermal safety studies, neither pimecrolimus nor tacrolimus induced phototoxicity or photosensitization.[20,37] After topical application of calcineurin inhibitors, serum concentrations are usually low or undetectable and repeated applications do not result in systemic accumulation.[56-60] High blood concentrations have only been reported in a few patients with compromised epidermal barriers[61] in whom topical calcineurin inhibitors are not indicated.

3.6 Clinical Data in Humans

3.6.1 Ultraviolet Light-Induced Pyridimine Dimers

Doelker and co-workers[62] examined the presence of UV light-induced dipyridimine dimers, which are believed to be an early step in skin carcinogenesis, in the epidermis of individuals with atopic dermatitis and healthy volunteers treated with topical pimecrolimus, the corticosteroid triamcinolone acetonide or vehicle only, and in untreated controls.

One hour after irradiation, pimecrolimus-treated epidermis showed less DNA damage than untreated epidermis, but there were no statistically significant differences between pimecrolimus, triamcinolone acetonide and vehicle. Furthermore, there were no significant differences in dipyridimine dimer levels between different treatments 24 hours after irradiation.[62]

3.6.2 Basal Cell Carcinoma and Squamous Cell Carcinoma

To elucidate nonmelanoma skin cancer (NMSC) induction, Naylor and coworkers [63] analysed data collected from 9813 adult and paediatric patients with atopic dermatitis who applied 0.03% or 0.1% tacrolimus ointment twice daily (mean duration 208 days; maximum observation period 1479 days) and were examined every 3 months. Ten White adult patients were diagnosed with basal cell carcinoma (BCC), three with SCC (12 of whom were >40 years of age). Based on 1718 patient-years of tacrolimus ointment exposure in patients ≥40 years of age, the calculated incidence of BCC or SCC did not suggest an increased risk of first BCC or SCC over that of a similarly aged US cohort.[63]

Margolis and coworkers[64] examined whether topical calcineurin inhibitor exposure was associated with an increased risk of NMSC. They used a questionnaire-based case-control study involving 5000 participants with atopic dermatitis (1000 with documented NMSC; 4000 controls). They found that the use of topical calcineurin inhibitors is not associated with an increased risk of NMSC (odds ratio [OR] adjusted for age, gender, previous NMSC, history of atopic dermatitis: 0.54; 95% CI 0.41, 0.69).

Ring et al.[65] were unable to document any kind of new neoplasia in a naturalistic multicentre, open-label study conducted in 2034 patients aged ≥3 months with mild to moderate atopic dermatitis receiving topical pimecrolimus twice daily until clearance of symptoms, for a treatment duration of up to 3 months.

3.6.3 Lymphoma

Hodgkin's disease, B-cell non-Hodgkin's lymphoma and T-cell non-Hodgkin's lymphoma (e.g. mycosis fungoides and Sézary syndrome) are part of the lymphoma group. In the US, the incidence of lymphoma ranges from around one case per 100 000 persons aged 0–19 years to 54 cases per 100 000 in patients over 50 years of age. The systemic use of immunosuppressive agents increases the risk of lymphoma, especially after transplantation.[66] The observed immunosuppression-related lymphomas

are generally B-cell non-Hodgkin's lymphomas and develop within the first 2 years after transplantation.[67]

Any association, irrespective of treatment, between atopic dermatitis and lymphoma is unclear. Some authors have found an increased risk of haematological malignancies in patients with dermatitis.[68,69]

In contrast, at least two studies have detected that dermatitis decreases the risk of non-Hodgkin's lymphoma.[70,71] Söderberg and co-workers[72] reported an association between dermatitis during childhood and non-Hodgkin's lymphoma in adulthood yielding a relative risk of 2.3 (95% CI 1.0, 5.3). Zhang et al.[73] detected an increased OR of T-cell non-Hodgkin's lymphoma among women with dermatitis (OR 2.5; 95% CI 1.1, 5.7).

As mentioned earlier, long-term continuous application of tacrolimus ointment and pimecrolimus cream in an experimental ethanol solution to the skin of mice was associated with the development of lymphoma (26- and 47-fold, respectively, higher exposures as measured by the AUC, than the maximum individual exposure ever measured in humans after topical treatment).[74]

Nevertheless, the skin of mice is more permeable than human skin, resulting in systemic effects after application of topical calcineurin inhibitors, whereas systemic effects in humans after topical application of calcineurin inhibitors are minimal, as previously outlined.[58,59]

Postmarketing surveillance of topical calcineurin inhibitors has identified occasional cases of lymphoma in users of topical calcineurin inhibitors alone or in combination with topical corticosteroids.[75]

A nested case-control study by Arellano and coworkers[74] investigated the association between the use of topical calcineurin inhibitors, topical corticosteroids, systemic corticoids and severity of atopic dermatitis, with the emergence of lymphoma in patients with atopic dermatitis. They found 249 cases of lymphoma in 293 253 patients, with 81 of these <20 years of age. The adjusted analysis yielded the following ORs (and 95% CIs): severity 2.4 (1.5, 3.8), systemic steroids 1.5 (1.0, 2.4), 'super potency' topical corticosteroids 1.2 (0.8, 1.8), 'low potency' topical corticosteroids 1.1 (0.7, 1.6), pimecrolimus 0.8 (0.4, 1.6), tacrolimus 0.8 (0.4, 1.7) and concomitant topical calcineurin inhibitors and topical corticosteroids 1.0 (0.3, 4.1).[74]

Lymphomas occurring in the setting of immunomodulatory or immunosuppressive therapy are characterized by often unusual location (e.g. soft tissue, joint spaces and lungs), polymorphous and pleomorphic large cells or Hodgkin's-like morphology, the presence of EBV genome in lymphoma cells, post-immunomodulatory therapy development of B-cell lymphomas and regressing lymphomas after withdrawal of immunomodulatory therapy in a significant percentage of cases.[30]

The clinical symptoms and histological analyses of lymphomas associated with the use of topical calcineurin inhibitors differ from those usually associated with PTLD or with lymphoma occurring in an immunocompromised person (e.g. EBV-positive B-cell lymphoma).[30,51]

Atopic dermatitis and cutaneous T-cell lymphomas share several similar clinical symptoms, which provide some challenges in establishing the appropriate diagnosis at beginning of treatment. It is therefore possible that initial cases of inflammatory skin alterations for which topical calcineurin inhibitors have been prescribed were cutaneous T-cell lymphomas. Topical calcineurin inhibitors should be avoided in this setting and not be used for more than 6 weeks if the patient fails to respond.[31]

4. Off-Label Use of Tacrolimus and Pimecrolimus

4.1 Cutaneous Disorders

Topical calcineurin inhibitors are in use off-label for numerous cutaneous disorders, including seborrheic dermatitis, dyshidrotic hand dermatitis, cheiropompholyx, allergic contact dermatitis, toxic contact dermatitis, perianal dermatitis, psoriasis, acne, rosacea, lupus erythematosus, balanitis, vulvitis, lichen planus, lichen sclerosus et atrophicus, vitiligo, Netherton syndrome, chronic graft-versus-host disease, lichen striatus, chronic actinic dermatitis,

postscabious dermatitis, lichen aureus, annular granuloma and cutaneous plasmocytoma.[76-97] Most of these reports are in the form of observational studies, characterized by a small number of participants and an absence of a prospective, randomized double-blind study design.

4.2 Reports of Carcinogenesis

A few case reports mention the detection of neoplasms after the use of topical calcineurin inhibitors in an off-label setting. Fischer and Bradford[98] reported a paraclitorial SCC in a 72-year-old woman who had a 30-year history of psoriasis involving the groin area and a 2-year history of hypertrophic vulvar lichen sclerosus. The vulvar lichen sclerosus was treated for several months with topical corticosteroids. After 3 weeks of treatment with pimecrolimus 1% cream, a SCC was verified by histopathological examination. As the authors mention, except for pimecrolimus application, several other risk factors for SCC formation have to be taken into consideration: long standing, poorly controlled lichen sclerosus, which has the potential to transform into SCC spontaneously, association of hypertrophic lesions, chronic inflammation as a result of an accompanying fixed drug eruption and advanced age. Unfortunately, the authors do not mention the treatment(s) that had been used for the psoriasis of the groin area in the previous 3 decades (which may have included, for example, topical psoralenes and UVA light, or systemic corticosteroids), which might be relevant to the observed SCC.

Langeland and Engh[99] describe a 57-year-old man with balanoposthitis of more than 2 years' duration. After 2 weeks of topical treatment with clobetasol cream 0.05% and 2.5 months of treatment with tacrolimus ointment 0.1%, a SCC of the glans was confirmed. The authors do not provide any information on the lesional therapy of the balanoposthitis before the first consultation in their office and do not mention any systemic therapies with immunosuppressants (e.g. corticosteroids) in earlier life. Furthermore, they do not discuss the contribution of genital inflammation on SCC emergence.

Becker and co-workers[100] described the case of a female (aged >55 years) with oral and genital lichen planus. The patient received systemic dapsone therapy with vitamin E and topical mometasone cream twice daily. After 1 year, dapsone therapy was discontinued and systemic acitretin (0.5 mg/kg bodyweight/day) was initiated. The use of acitretin was abandoned 6 weeks later because of the elevation of serum lipids. Next, systemic dexamethasone 100 mg/day for 3 consecutive days every 4 weeks (three cycles) showed no improvement. Afterwards, tacrolimus 0.1% ointment twice daily resulted in pain relief after a few weeks of application. After about 36 months of more or less permanent topical use of tacrolimus, a SCC located at the tongue was confirmed by biopsy.

Interestingly, the authors reported a reduction in Bax (a proapoptotic member of the Bcl-2 family) expression as a marker for p53 activation in epithelial cells of the mucosa and in carcinoma cells treated with topical tacrolimus, suggesting an influence on proteins (such as p53), which are involved in cancer-signalling pathways.[100] As the authors state, the large number of different agents in use in this case report make it somewhat difficult to speculate on the carcinogenic potential of each single agent.

5. Discussion

Topical calcineurin inhibitors have been in clinical use for less than a decade; therefore, understanding of their long-term potential for the induction of neoplasia is limited. Nevertheless, given the enormous level of exposure of patients of all age groups with atopic dermatitis to tacrolimus and pimecrolimus, the potential risk of malignancy seems to be low. To rule out any potential risk for patients with atopic dermatitis in the future, investigation through longer-term trials is required,[31] and phase IV postmarketing surveillance studies with a large sample size and an extended follow-up of patients with atopic dermatitis using topical calcineurin inhibitors must be conducted.

Novartis, the marketing authorisation holder (MAH) of pimecrolimus, has launched a long-term

prospective observational study in children (Pediatric Elective Registry) and a retrospective case-control study on skin cancer of 5000 patients with atopic dermatitis selected from the University of Pennsylvania NMSC Registry.[29]

Astellas, the MAH of tacrolimus has initiated, as part of its phase IV commitment, a multinational, observational cohort study of 8000 patients with atopic dermatitis, with direct contact with the patient every 6 months, an annual physical examination and a biannual dermatological examination. Study endpoints include systemic malignancies (Hodgkin's disease or non-Hodgkin's lymphoma) and skin cancer.[29] As reported in February 2007, there have been no reports of malignancies to date in 1000 patients enrolled (average age of enrollment 7.5 years; average duration of atopic dermatitis 5.5 years; mean cummulative tacrolimus ointment administration 2.5 years per patient [range 0.00–10.25 years]).[101]

Furthermore, long-term controlled studies in patients <2 years of age, patients with non-atopic dermatitis inflammatory disorders of the skin, as well as comparative studies with different potencies of corticosteroids in patients with atopic dermatitis are needed.[31]

In this context, Paul and co-workers[59] reviewed the safety and tolerability of 1% pimecrolimus cream in 1133 patients 3–23 months of age with mild to severe atopic dermatitis treated for up to 2 years. The study population was drawn from four pharmacokinetic studies and six clinical trials conducted among these patients. The authors reported neither signs of immunosuppression (with the risk of immunosuppression-related lymphoma occurrence) nor cases of skin malignancies.

At present, available data support the use of topical calcineurin inhibitors for atopic dermatitis, as outlined in the manufacturer's prescribing information. Until long-term study data are available, it seems prudent that topical calcineurin inhibitors should:

1. not be used in children <2 years of age;
2. not be used continuously for >6 weeks, with an application-free period of up to 2 weeks;
3. be avoided in immunocompromised patients (e.g. post-organ transplantation);
4. be avoided in patients with neoplasia (e.g. lymphoma);
5. be avoided in patients with skin disorders where there is an increased risk of systemic absorption (e.g. Netherton syndrome);
6. be accompanied by encouraging patients to apply broad-spectrum sun block daily to all sunlight-exposed skin.

Since the FDA added a 'black box' warning based mainly on preclinical data, but did not change the indications for use, physicians, parents of children with atopic dermatitis and adult patients with atopic dermatitis have to be informed of the benefits and risks associated with topical calcineurin inhibitors in an ongoing process.[31] While no further data are available on safety issues, tolerability and efficacy in individual patients will remain the most important aspect of the choice of treatment.[51]

At present, topical calcineurin inhibitors are without doubt valuable parts of the therapeutic armamentarium in the management of atopic dermatitis.

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