

The safety of tacrolimus ointment for the treatment of atopic dermatitis: a review

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Summary

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Conflicts of interest

M.H.A.R. has been involved in clinical trials with tacrolimus ointment, has acted as a consultant for Fujisawa (now Astellas Pharma) and has undertaken research work supported by Fujisawa (now Astellas Pharma).

Tacrolimus ointment is a topical calcineurin inhibitor (TCI) that was developed specifically for the treatment of atopic dermatitis (AD). It is one of the most extensively tested dermatological products, with more than 19 000 patients (including approximately 7600 children) having participated in the tacrolimus ointment clinical development programme. Recent regulatory reviews have focused on the potential risk of malignancy with TCIs, based on their mode of action and the effects of systemic tacrolimus when given to transplant recipients. Studies have shown, however, that the systemic absorption of tacrolimus when applied topically is very low, with blood concentrations being below the level of quantification in most patients. Moreover, TCIs are not associated with a decrease in immunocompetence in the skin and there is no increase in the incidence of infections with long-term treatment. More than 5.4 million prescriptions for tacrolimus ointment have been issued worldwide, with no evidence of an increased risk of malignancy in adults or children compared with the general population. Similarly, epidemiological studies have failed to demonstrate an increased incidence of skin cancer in patients using TCIs. The most common adverse events (AEs) that occur with tacrolimus ointment treatment are transient application-site reactions, such as burning or pruritus. These complications are related to disease severity, and decrease in frequency over time as AD improves. The incidence of nonapplication-site AEs does not increase with long-term treatment, and most such events occurring in clinical trials were considered to be unrelated to therapy. Although it is important that clinicians are aware of the recent changes in product labelling, extensive clinical trials continue to show that tacrolimus ointment is well tolerated, and is generally an effective therapy for suitable patients with AD.

Tacrolimus ointment (Protopic® 0.03% and 0.1% ointment; Astellas Pharma GmbH, Munich, Germany) is a topical calcineurin inhibitor (TCI) and was the first of this novel class of topical preparations to be developed specifically for the treatment of atopic dermatitis (AD). Consequently, it is the primary focus of this review. Compared with topical corticosteroids, which have been the mainstay of AD therapy for the past 50 years, the TCIs have a more selective mode of action. Whereas corticosteroids exert a wide variety of cellular effects because they alter the transcription of numerous genes in a number of different cell types,¹ TCIs specifically target key immunological mechanisms that underlie the pathogenesis of AD, and act primarily on T cells in lesional skin.²

Tacrolimus, a macrolide lactone, was originally isolated in Japan in the mid-1980s and is a natural product of the fungus-like bacterium *Streptomyces tsukubaensis*.³ The immuno-

suppressant properties of tacrolimus were identified in 1987³ and an oral preparation (Prograf®; Astellas Pharma Ltd, Staines, U.K.) was first approved in Japan in 1993 for liver transplantation (primary and rejection). Tacrolimus was first incorporated into an ointment for topical use in the 1990s, and was first approved for the treatment of AD in Japan in 1999, with approvals following for the U.S.A. in 2000 and for the European Union in 2002.

Tacrolimus acts primarily on T cells in affected skin, preventing T-cell activation and suppressing the release of pro-inflammatory cytokines.⁴ It blocks T-cell activation by binding to the cytosolic FK-506 binding protein-12 to form a complex that inhibits the activity of the enzyme calcineurin.⁴ In addition, tacrolimus specifically affects other immune cells that are involved in the pathogenesis of AD, blocking cytokine production and release in mast cells, eosinophils and basophils,

inhibiting T-cell activation by antigen-presenting dendritic cells, and reducing the number of inflammatory dendritic epidermal cells in AD lesions.^{5–9} When applied to the skin, tacrolimus ointment temporarily modulates aberrant local immune responses to relieve the signs and symptoms of AD.

As the first real alternative to topical corticosteroids in the treatment of AD, the introduction of the TCIs represented an important therapeutic advance. The clinical development programme for tacrolimus ointment is one of the most extensive that has ever been conducted in dermatology, with more than 19 000 patients (including approximately 7600 children) having participated in the tacrolimus ointment clinical development programme.⁹ In total, 16 phase III studies have been conducted in North America, Europe and Japan,^{10–23} with four phase IV studies comparing tacrolimus ointment with the other commercially available TCI, pimecrolimus 1% cream (Elidel®; Novartis Pharma AG, Basel, Switzerland; Table 1).^{24,25} Overall, the tacrolimus ointment clinical trial programme has demonstrated that both strengths are effective and well tolerated for the treatment of mild to severe AD in adults and children, including those with extensive disease.

Because systemic immunosuppression is associated with an increased risk of nonmelanoma skin cancer (NMSC), there is a theoretical possibility that topical drugs such as tacrolimus ointment and pimecrolimus cream may increase the risk of skin cancer. Although there is no evidence of a causal link

between TCIs and skin cancer, these agents have recently been reviewed by regulatory agencies in the U.S.A. and the European Union, and changes have been made to the product labelling. This article reviews these regulatory changes and also summarizes the safety and tolerability profile for tacrolimus ointment.

Tacrolimus ointment: recent regulatory reviews

Reviews of the TCI class of drugs were initiated early in 2005 by the Pediatric Advisory Committee of the U.S. Food and Drug Administration (FDA) and the Committee for Medicinal Products for Human Use/European Medicines Agency (CHMP/EMA) in Europe. The reviews were commissioned as a result of rare reports of cancer in patients treated with TCIs,²⁶ the theoretical risk of malignancy based on the mechanism of action of TCIs, data from animal studies with sustained high exposure²⁶ and data from transplant recipients treated with systemic calcineurin inhibitors.²⁷

In the U.S.A., several changes were made to the prescribing information (PI) for the TCIs as a result of the FDA review. These included changes to the 'Indications and Usage' section, the addition of a 'Warnings' section – with a boxed warning – and the addition of a 'Medication Guide' to be dispensed to patients with each prescription, as well as general updates to include recent pharmacokinetic and adverse event (AE) data. As a result of these changes, tacrolimus ointment is indicated as second-line therapy for the short-term and noncontinuous chronic treatment of moderate to severe AD in nonimmunocompromised adults and children who have failed to respond adequately to other topical prescription treatments for AD, or when those treatments are not advisable. The boxed warning notes that long-term safety of TCIs has not been demonstrated and that, although a causal relationship has not been established, rare cases of malignancy (e.g. skin and lymphoma) have been reported in patients treated with TCIs, including tacrolimus ointment.²⁸ As a result, the revised PI states that 'continuous long-term use of TCIs, including Protopic Ointment, in any age group should be avoided, and application limited to areas of involvement with atopic dermatitis'. In addition, the PI states that tacrolimus ointment is not indicated for use in children who are younger than 2 years of age or in immunocompromised individuals, and that only the 0.03% formulation of tacrolimus ointment is indicated for use in children of 2–15 years of age.

The safety review that was conducted by the CHMP/EMA concluded that the risk–benefit profile of tacrolimus ointment is still favourable, although some amendments to the summary of product characteristics (SmPC), which are broadly similar to those changes made to the U.S. PI, have been made. The indication for tacrolimus ointment was clarified to note that tacrolimus ointment should be prescribed for the treatment of moderate or severe AD in patients who are not adequately responsive to or are intolerant of conventional therapies, such as topical corticosteroids. As in the U.S.A., tacrolimus ointment is not recommended for use in children aged under 2 years or

Table 1 Summary of clinical trials with tacrolimus ointment

Study type	Study details
Vehicle-controlled	6-week paediatric ^a trial (n = 317) ²¹ 6-week adult ^a trial (n = 300) ⁶⁹ Two 12-week adult trials (n = 632) ^{10,11} 12-week paediatric ^a trial (n = 351) ¹²
Comparison vs. corticosteroid ^b	1-week adult trial (n = 151) ²³ 3-week adult trial (n = 181) ²³ 3-week adult trial (n = 570) ¹⁵ 3-week paediatric ^a trial (n = 560) ¹⁶ 3-week paediatric ^a trial (n = 624) ¹⁷ 6-month adult trial (n = 972) ¹⁸
Comparison vs. pimecrolimus cream	6-week paediatric ^a trial (n = 225) ²⁴ 6-week paediatric ^a trial (n = 425) ²⁴ 6-week adult trial (n = 413) ²⁴ 6-week paediatric ^c trial (n = 141) ²⁵
Long-term, open-label, noncomparative	12-month adult trial (n = 316) ¹³ 12-month paediatric ^a trial (n = 255) ¹⁴ 24-month adult trial (n = 568) ^d Up to 29.5-month paediatric ^a trial (n = 466) ²² Up to 2-year adult and paediatric ^a trial (n = 7923) ¹⁹ Up to 4-year adult and paediatric ^a trial (n = 799) ²⁰

^aChildren aged 2–15 years. ^bHydrocortisone acetate, hydrocortisone butyrate, betamethasone valerate or alclometasone dipropionate ointments. ^cChildren aged 2–17 years. ^dAstellas Pharma, data on file.

in immunocompromised individuals. In addition, treatment with tacrolimus ointment should be initiated only by physicians who have experience in the diagnosis and treatment of AD. The SmPC also states that treatment should be intermittent and not continuous, and application should be limited to the areas of involvement with AD. The SmPC notes that cases of malignancies, including cutaneous and other types of lymphoma, and skin cancers have been reported in patients receiving tacrolimus ointment, and that TCIs should not be used on potentially malignant or premalignant skin lesions. The SmPC also adds that the presentation of cutaneous T-cell lymphoma can sometimes be misdiagnosed as AD.

Topical calcineurin inhibitors and theoretical cancer risk

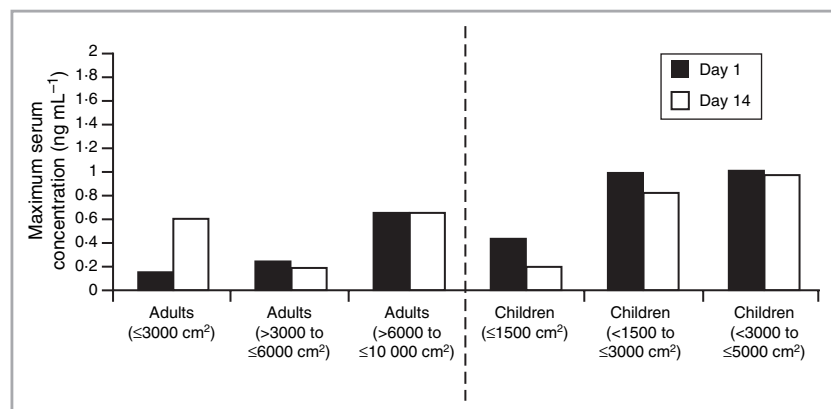
Systemic tacrolimus exposure with use of tacrolimus ointment

In studies to date, all the data show that there is a low risk of systemic exposure to tacrolimus, with consistently low or no systemic absorption, after topical application.^{11–13,15,29–37} *In vitro* studies that were conducted to evaluate the rate and extent of percutaneous tacrolimus absorption through isolated human skin preparations showed that there was little or no absorption of tacrolimus after a single application of tacrolimus 0.03% or 0.1% ointment, or of a noncommercial 0.3% formulation.³⁸ Overall, pharmacokinetic studies have shown that systemic exposure to tacrolimus 0.3%, 0.1% or 0.03% ointment is low, with blood concentrations being below the level of quantification (0.5 ng mL^{-1}) in the majority of patients.^{29,30,33,34} For example, an open-label pharmacokinetic study was carried out in 39 patients aged 5–75 years with moderate to severe AD.³⁰ Tacrolimus 0.3% ointment was applied to areas of 50–5000 cm^2 on the face or trunk/limbs, and patients received 14 applications over 8 days. Systemic exposure to tacrolimus was very low and the mean maximum blood concentrations were $0.1\text{--}1.9 \text{ ng mL}^{-1}$ in children (aged 5–11 years) and $0.2\text{--}3.5 \text{ ng mL}^{-1}$ in adults (aged 14–75 years).³⁰ More recent pharmacokinetic studies in adults³⁴ and children^{33,35,36} have shown similar results (Fig. 1), with

no evidence of systemic accumulation after repeated application of tacrolimus 0.1% ointment. The pharmacokinetics of tacrolimus ointment have also been evaluated in children who are younger than 2 years of age, with no increased blood tacrolimus levels being evident after 1 month of routine treatment.²⁹ More recently, a pharmacokinetic study of tacrolimus 0.03% ointment has been carried out in infants aged 3–24 months, showing that systemic exposure to tacrolimus was minimal, although highly variable between patients.³⁷ Overall, 97% of the blood samples contained tacrolimus concentrations below 1 ng mL^{-1} , while 20% contained concentrations below the lower limit of quantification (0.025 ng mL^{-1}). Systemic exposure tended to increase with increasing body surface area treated, and was approximately 3% of that recorded in paediatric transplant patients receiving oral tacrolimus.³⁷ These studies have also demonstrated that tacrolimus absorption decreases over time, in parallel with clinical improvements in AD as the skin heals, even when the amount of ointment that is applied remains constant.^{33,34} It seems likely that this is the result of improvement in skin barrier function.

Throughout the tacrolimus ointment clinical trial programme, no AEs have been associated with detectable blood concentrations of tacrolimus. Of over 2000 patients in whom systemic exposure was evaluated during clinical development worldwide, only isolated cases of blood levels above 5 ng mL^{-1} were recorded. Of these, two patients had a maximum of 20 ng mL^{-1} after whole-body application with 10 g of tacrolimus 0.1% ointment in an early Japanese study.³⁹ All of these exceptional cases, in which extremely large amounts of the ointment were applied, were single, transient occurrences that were not associated with AEs. Of the two Japanese patients, blood concentration in one decreased in a time-dependent manner, while the concentration in the second patient decreased as the skin condition improved.³⁹ Treatment with tacrolimus 0.1% ointment in three patients with Netherton syndrome – a disorder that is characterized by congenital erythroderma and skin barrier dysfunction – resulted in high blood tacrolimus concentrations.⁴⁰ Maximum blood concentrations in the patients were 37.2 , 23.0 and 10.2 ng mL^{-1} , respectively, but none of the patients experienced systemic AEs. It is clear, however, that diseases such as

Fig 1. Mean maximum blood tacrolimus concentrations in adults³⁴ and children³³ receiving tacrolimus 0.1% ointment, stratified by body surface area affected.



Netherton syndrome that involve skin barrier defects are more likely to allow high absorption of topically applied medications, even those with a relatively large molecular size, such as tacrolimus. Indeed, the SmPC for tacrolimus ointment specifically advises against using it in patients with this condition.

Immunocompetence

Because the TCIs modulate local immune responses in the skin, the use of tacrolimus ointment could, in theory, increase the risk of cutaneous infections as a result. The considerable amount of data that has been gathered to date shows that, overall, the TCIs are not associated with an increased risk of skin infections with long-term use.

In vitro studies have shown that tacrolimus inhibits lymphocyte proliferation in a dose-dependent manner, ranging from 9.2% inhibition at 0.1 ng mL^{-1} to 55% at 5 ng mL^{-1} .^{41,42} The potential effect of tacrolimus 0.1% on immune responses was evaluated using the recall-antigen test (Multitest CMI; Institut Mérieux, Lyon, France) to assess delayed hypersensitivity reactions as an indirect measure of cellular immune response in a cohort of patients from a noncomparative, 12-month study in adults.¹³ Skin testing was carried out at baseline and after 6 and 12 months of treatment using an eight-pronged applicator containing one vehicle control and seven bacterial and fungal antigens (tetanus toxoid; diphtheria toxoid; *Streptococcus*; tuberculin, old; *Candida*; *Trichophyton* and *Proteus* antigens). Reactions were quantified by determining the mean of the maximum diameter and the diameter that is orthogonal (at right angles) to it. A positive test reaction was one of at least 2 mm in diameter, and the sum of the diameters from positive reactions yielded the 'Mérieux score'. Tacrolimus ointment was found to have no measurable effect on cellular immunity, with no change in the level of immunocompetence from baseline to months 6 and 12 (Fig. 2).¹³ The mean $\pm$ SD number of positive antigens per patient was 1.3 ± 1.2 at day 1, 1.5 ± 1.3 at month 6 and 1.8 ± 1.4 at month 12. The proportion of patients with no positive reactions was also similar over time: 28 patients (33%) at day 1, 16 patients (26%) at month 6 and

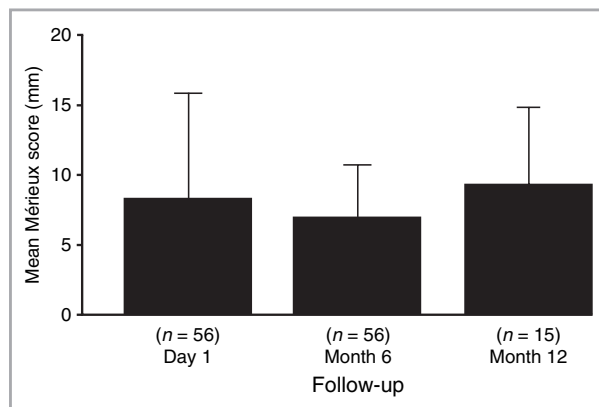


Fig 2. Recall-antigen test results (mean $\pm$ SD) from a 1-year study of tacrolimus ointment in adults and children.¹³

five patients (25%) at month 12. These results also showed that the patients had depressed cell-mediated immunity at baseline, which is consistent with previously published observations that cell-mediated immunity is impaired in patients with AD.⁴³ Overall, various immunological parameters are affected in patients with AD: the relative and absolute numbers of circulating T cells are reduced; lymphocyte responses to mitogens are impaired; the cytotoxic potential of several cell types, such as monocytes and natural killer cells, is decreased; and chemotactic responsiveness is reduced.⁴⁴ There are few published data on the effect of tacrolimus ointment on these parameters, although studies in a mouse model of contact hypersensitivity have shown that tacrolimus ointment dose-dependently suppresses oxazolone-induced lymph node cell proliferation.⁴⁵ This effect was correlated with decreased epidermal mRNA expression of numerous cytokines, including interleukin (IL)-1 α , IL-1 β , tumour necrosis factor- α and interferon (IFN)- γ , as well as decreased IL-12 p35 and p40 mRNA expression in lymph node cells. In addition, production of Th1 (IFN- γ and IL-2) and Th2 (IL-4) cytokines was impaired after tacrolimus ointment treatment, and the population of epidermis-infiltrating CD4+ T cells was decreased.⁴⁵ Tacrolimus ointment treatment may be associated with improvement in responses to recall antigens.⁴⁶ In a study involving patients with moderate to severe AD, approximately one third of the patients were anergic at baseline. After 1 year of intermittent therapy with tacrolimus 0.1% ointment, only one patient remained anergic, and positive reactions were observed when this patient was evaluated at year 3. Overall, these results indicate that tacrolimus ointment use does not compromise cellular immune responses.

Clinical improvement after tacrolimus ointment treatment has been shown to lead to reduced levels of *Staphylococcus aureus* being present on the skin,^{47,48} again indicating that tacrolimus ointment does not affect cellular immune responses. In one study, 40 lesional skin sites that were positive for *S. aureus* colonization were identified in 11 patients (mean age 23.2 years).⁴⁷ All patients stopped topical or systemic antibiotic treatment 2 weeks before starting treatment with tacrolimus 0.1% ointment for 3 weeks. *Staphylococcus aureus* colonization density was measured as the number of agglutination-positive colony-forming units (CFU) sampled on a mannitol salt agar contact plate at baseline and at days 3, 7, 14 and 21 of tacrolimus treatment. Of 151 samples taken, 139 (92.1%) could be evaluated. As a control, 11 positive lesional sites in 11 patients were treated with vehicle alone. Tacrolimus ointment treatment was associated with an overall decrease of *S. aureus* colonization on lesional skin ($P < 0.05$) from day 7 onwards. Similar results were obtained in a long-term study of adults with AD in which 19 patients were treated for 6 months and nine were treated for 12 months with tacrolimus 0.1% ointment.⁴⁸ By the end of week 1, *S. aureus* colonization had decreased significantly: the median decrease in CFU cm^{-2} compared with baseline was 59.1 at week 1 ($P = 0.012$), 102.4 at month 6 ($P < 0.001$) and 234.9 at month 12 ($P = 0.008$). The decreases in staphylococcal colonization

were coupled with significant improvements in clinical AD scores in both studies. As tacrolimus does not have inherent antibacterial properties, it seems probable that the effect on *S. aureus* colonization is the result of lesion healing and improvements in skin barrier function. Similar effects on *S. aureus* colonization were seen in a second study, in which 65 Korean patients with moderate to severe AD received tacrolimus 0.03% ointment twice daily for 4 weeks.⁴⁹ Colonization was significantly decreased with tacrolimus treatment from week 1 compared with baseline.

The risk of cutaneous infections in patients who participated in three vehicle-controlled studies and two 12-month, non-comparative studies ($n = 1554$)^{10–14} was analysed retrospectively.⁵⁰ In this analysis, there were no significant differences between the number of bacterial, viral or fungal infections with tacrolimus ointment vs. vehicle in adults or children. The 12-week adjusted incidences of all cutaneous infections in patients who were treated with vehicle or tacrolimus 0.03% or 0.1% ointment, respectively, were 18.0%, 24.8% and 17.7% in adults and 20.9%, 19.6% and 23.6% in children.⁵⁰ Long-term (up to 1-year) use of tacrolimus ointment yielded no detectable increase in the risk of cutaneous infections. In addition, no increase in the risk of cutaneous infections has been seen either in adults ($n = 4372$) or in children ($n = 4816$) in three long-term studies of up to 4 years' duration.^{19,20,22}

Additional evidence supporting a lack of effect on immuno-competence comes from a recent short-term paediatric study that investigated the effect of tacrolimus ointment on immune responses in 26 children (aged 2–12 years) after administration of the pneumococcal polysaccharide vaccine (Pneumovax® 23).⁵¹ Twenty-three children who were treated with tacrolimus 0.03% ointment for 7 weeks and received the vaccine at week 3 were evaluable. All of the children developed protective antibody responses to at least eight serotypes, whereas 70% had protective titres to all 12 serotypes (Fig. 3). Similar results were seen in a 7-month, double-blind, active-controlled study in 132 children who received the meningo-

coccal serogroup C vaccine during treatment with tacrolimus ointment or a hydrocortisone regimen, compared with 44 children without AD.⁵² The response rate (defined as patients with a serum bactericidal antibody titre ≥ 8) was 97.5% in the tacrolimus ointment group compared with 99.1% and 97.7% in the hydrocortisone ointment and control groups, respectively. Tacrolimus ointment had no adverse effects on immediate response to vaccination, generation of immune memory, humoral immunity or cell-mediated immunity.⁵² Overall, the results of these studies indicate that tacrolimus ointment does not affect humoral immune responses or the distribution of lymphocyte subsets, nor does it interfere with the antibody response to vaccination.

Carcinogenicity

Long-term carcinogenicity studies in mice and rats do not demonstrate any evidence of malignant change after topical administration of tacrolimus ointment.²⁸ Additional preclinical experiments have evaluated the photocarcinogenic potential of tacrolimus ointment (Astellas Pharma, data on file). In these photocarcinogenicity studies, hairless mice were exposed to levels of ultraviolet (UV) radiation that were sufficient to induce tumours in all animals, including untreated control animals, to provide a comparison of the time to tumour onset with different treatments. This experimental model is used in the development of many dermatological drugs, although its relevance to humans has not been determined. In these studies, vehicle treatment reduced the time to skin tumour development, probably because the vehicle enhanced penetration of UV radiation into the skin (Astellas Pharma, data on file). Tacrolimus 0.03% or 0.1% ointment did not further reduce the time to tumour formation: there were no significant differences between tacrolimus 0.03% or 0.1% ointment and vehicle. Higher tacrolimus ointment concentrations than those commercially available reduced the time to tumour development compared with vehicle, but this was probably the result of a systemic effect and is unlikely to be clinically relevant (Astellas Pharma, data on file). Similar results were obtained in separate studies with pimecrolimus cream.⁵³ Conversely, a recent study has shown that TCIs can prevent DNA photodamage in hairless mice as the result of a filtering effect of both the vehicle and the active ingredient.⁵⁴

In a carcinogenicity study in CD-1 mice, Jiang et al.⁵⁵ investigated the effect of topical tacrolimus on the formation of skin papillomas induced by 7,12-dimethylbenz[a]anthracene and promoted by 12-O-tetradecanoylphorbol-13-acetate (TPA). In this study, 7- to 8-week-old female mice were treated for 22 weeks either with TPA alone or with topical tacrolimus applied 15 min before TPA. Topical application of tacrolimus resulted in a dose-related inhibition of tumour formation, with 1 μmol almost completely inhibiting tumour formation. In addition, topical tacrolimus resulted in a dose-related inhibition of epidermal ornithine decarboxylase induction and skin inflammation caused by TPA, both of which are thought to be related to skin tumour promotion. Overall, the authors

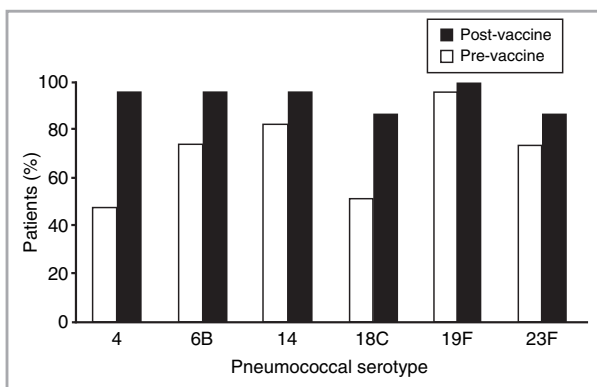


Fig 3. Percentage of children with protective titres to pneumococcal serotypes after vaccination during treatment with tacrolimus ointment.⁵⁰

concluded that tacrolimus ointment had an inhibitory effect on TPA-mediated tumour promotion.⁵⁵ In contrast to this study, one recent report has suggested that topical tacrolimus can accelerate carcinogenesis in mouse skin.⁵⁶ In this study, approximately 8 cm² of the shaved dorsal skin of 7-week-old female CD-1 mice were treated with a tumour initiator, followed by a tumour promoter or acetone, with or without topical tacrolimus. In the mice that were treated with tacrolimus, there was an increase in the number of skin tumours compared with mice treated with the promoter or acetone alone. More than 90% of the tumours were, however, papillomas, which are rare and benign in humans. In addition, the amount of tacrolimus ointment applied in this study (equivalent to 100 g daily) is more than sixfold greater than the standard dose used in clinical practice (0.1 g per 100 cm² daily), and the relevance of the model to the carcinogenic process in human skin has not been established. It would appear, therefore, that these findings are unlikely to be clinically relevant.

To date, more than 5.4 million prescriptions for tacrolimus ointment have been dispensed worldwide and there is no evidence to suggest that treatment is associated with an increased risk of cancer in either adults or children.⁵⁷ Indeed, shortly before the FDA and EMEA reviews of TCIs, a position statement was issued by The European Dermatology Forum,⁵⁸ which is a nonprofit professional organization made up of leading dermatologists from most European countries. Based on conclusions drawn from critical appraisal of available data on TCIs, both preclinical and clinical, the statement noted that data from rodent trials are of limited application to humans and that there is no conclusive evidence from such studies to indicate that long-term application of TCIs is photocarcinogenic.⁵⁸

Since the FDA and EMEA reviews were initiated, individual physicians and professional groups worldwide have voiced their concerns, via published articles, that these claims of malignancy have no evidence to support them.^{9,59–64} Of note, the incidence of NMSC was analysed in 9813 patients (aged 2 to ≥60 years) who were treated with tacrolimus ointment in clinical trials in the U.S.A.⁶⁵ Overall 1718 patient-years of follow-up after topical tacrolimus exposure were accrued during these studies. The longest period of observation was 1479 days, and 300 patients were followed for over 36 months. Thirteen patients, all adults, were diagnosed with NMSC during the study period: 12 patients aged >40 years and one patient <40 years (median age 61 years). Four patients had a prior history of NMSC and two patients had previously been exposed to systemic immunosuppressants (cyclosporin and methotrexate) or phototherapy. None of the cases of NMSC was considered by the investigator to be related to tacrolimus ointment.⁶⁵ In the U.S. Physician's Health Study, patients aged >40 years who received tacrolimus ointment had an incidence of NMSC estimated to be between 361 and 1217 per 100 000 patient-years, while an incidence of 533 per 100 000 patient-years for first NMSC in healthy individuals was reported.⁶⁶ This suggests that tacrolimus ointment does not increase the risk of NMSC in patients

with AD. These findings have been confirmed by a recent report from a case-control study, which has suggested that TCIs may even protect against skin cancers.⁶⁷ Between 2002 and 2005, data were collected from 1000 patients with NMSC and 4000 controls. Of these, 14.4% of patients with NMSC and 30.7% of controls were exposed to TCIs (age-adjusted odds ratio, OR 0.54). Overall, TCIs had an apparent protective effect, which increased with an increasing number of tubes of ointment or cream used.⁶⁷ The effects of TCIs and corticosteroids on the incidence of lymphoma have also been investigated in a case-control study involving 293 253 patients with AD.⁶⁸ Overall, severity of AD was the main factor associated with an increased risk of lymphoma (OR 2.4). There was no association between lymphoma and pimecrolimus cream (OR 0.8) or tacrolimus ointment (OR 0.8), nor between lymphoma and use of topical corticosteroids (low potency, OR 1.1; high potency, OR 1.2).⁶⁸

Although it seems there are no convincing data to support the possibility that TCIs increase the incidence of cutaneous malignancies, patients should be informed of the risk-benefit hypothesis with tacrolimus ointment and advised that an exposure period of 15 years may have to elapse before such a suggestion can be completely disregarded.

Adverse events and tacrolimus ointment

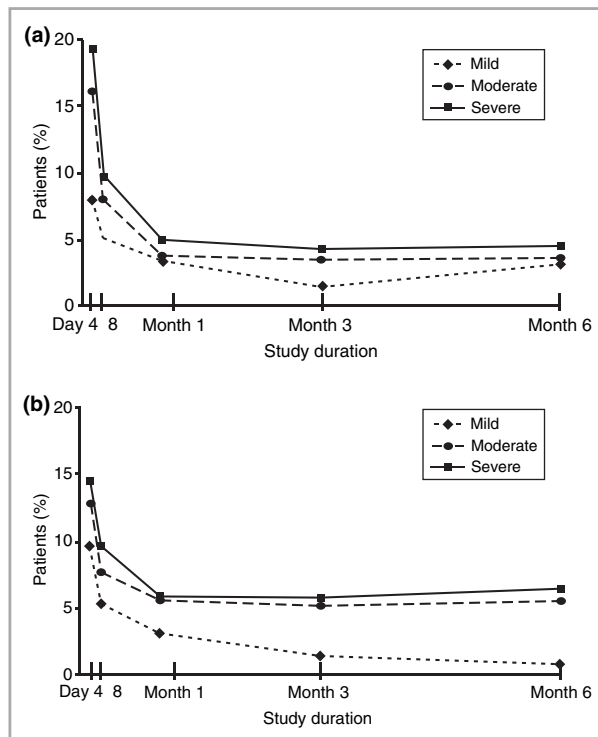
Common adverse events

The most common AEs associated with tacrolimus ointment are application-site reactions, such as burning sensations and pruritus (Table 2). These are usually mild to moderate in intensity and transient, lasting 15–20 min and occurring during the first few days of treatment (Fig. 4).^{11–22} Application-site events resolve rapidly as the skin heals, and improvement in disease status is associated with a decrease in the incidence and severity of the burning sensation and other local AEs.¹³ The burning sensation, which is generally experienced as a feeling of warmth or heat, and includes events such as pain, stinging and soreness, has been reported by 18–59% of patients in clinical trials. Application-site pruritus and erythema were reported by at least 10% of patients in clinical studies²⁸ and, as with burning and pruritus, erythema was also generally mild to moderate in severity and transient. In general, the burning sensation, pruritus and erythema resolved within 1 week of initiating tacrolimus ointment and occurred more frequently in adults than in children (Table 2).

In the clinical studies, the incidence of application-site AEs seemed to be related to the severity of disease: compared with patients with less severe disease, more patients with severe disease or with >75% of their body surface area affected reported these events.^{11,17,19} In addition, a retrospective analysis of two long-term studies demonstrated that application-site reactions were reported half as often in patients with mild disease compared with severely affected patients.⁶⁹ In a pooled analysis of results from two randomized, double-blind studies – one in adults and one in children – in patients with mild to

Table 2 Incidence of common application-site adverse events in phase III trials^{11–22}

	Vehicle (%)	Tacrolimus 0.03% ointment (%)	Tacrolimus 0.1% ointment (%)	Corticosteroid ^a (%)
Burning sensation				
Children (2–15 years)	29	18–43	19–34	7–15
Adults (≥16 years)	26	45–46	23–59	13–14
Pruritus				
Children (2–15 years)	27	13–41	11–32	8–16
Adults (≥16 years)	37	20–46	15–46	10–16
Erythema				
Children (2–15 years)	19	2–8	1–8	1–2
Adults (≥16 years)	20	2–25	4–28	1–4

^aHydrocortisone acetate and/or hydrocortisone butyrate ointments.**Fig 4.** Prevalence of (a) pruritus and (b) skin burning over time in patients with mild, moderate or severe atopic dermatitis.¹⁹

moderate disease ($n = 617$), no significant differences were found between the tacrolimus ointment and vehicle treatment groups in the incidence of burning or stinging.⁷⁰ Moreover, the incidences of itching and erythema were higher with vehicle than with tacrolimus ointment.

In comparative studies vs. corticosteroids, the incidences of burning sensation and, to a lesser extent, pruritus were significantly higher with tacrolimus ointment than with vehicle or corticosteroids (Table 2). For example, in a double-blind, 3-week study comparing tacrolimus 0.03% ($n = 193$) and 0.1% ($n = 191$) ointments with hydrocortisone butyrate 0.1% ointment ($n = 186$) in adults with AD, burning sensation and pruritus were the only AEs that had a higher incidence with

tacrolimus ointment than with hydrocortisone butyrate ($P < 0.05$; Table 3).¹⁵ Similar results were seen in a 3-week double-blind study in 560 children (aged 2–15 years) that compared tacrolimus 0.03% ($n = 189$) and 0.1% ($n = 186$) ointments with hydrocortisone acetate 1% ointment ($n = 185$); in this study, burning sensation was the only AE that had a significantly higher incidence with tacrolimus ointment than with hydrocortisone acetate (Table 3; $P < 0.05$).¹⁶

Common nonapplication-site AEs that are seen during treatment with tacrolimus ointment include influenza-like symptoms, headache, allergic reaction and asthma.²⁸ These occur in similar numbers of patients treated with topical corticosteroids. Additional AEs reported by 1–10% of patients include skin infection, skin tingling, folliculitis, acne, alcohol intolerance (defined as facial flushing or facial skin irritation after alcohol intake), hyperaesthesia and herpes simplex.²⁸

AEs, including application-site reactions, rarely lead to treatment discontinuation with tacrolimus ointment. In nine phase III studies of up to 12 months' duration, the percentage of patients prematurely discontinuing treatment because of AEs was 1.4–6.2% (mean 3.6%) with tacrolimus 0.03% ointment and 1.6–5.3% (mean 3.3%) with tacrolimus 0.1% ointment.^{10–18} The most common AEs leading to treatment discontinuation included burning sensation, pruritus, erythema, skin infection and herpes simplex infection.

The profile of AEs seen in patients treated with tacrolimus ointment is similar to that seen with pimecrolimus cream, particularly in those studies in which the two agents are compared directly.^{24,25} Two studies have compared tacrolimus 0.1% ointment with pimecrolimus 1% cream, one in adults with mild to very severe AD ($n = 413$) and one in children (aged 2–15 years) with moderate to very severe AD ($n = 225$); a third study has compared tacrolimus 0.03% ointment with pimecrolimus 1% cream in children (aged 2–15 years) with mild AD ($n = 425$).²⁴ In a combined analysis of all three studies, the incidence of cutaneous AEs with tacrolimus ointment ($n = 530$) was similar to that with pimecrolimus cream ($n = 533$) (Fig. 5), with the only statistically significant difference between the treatments being a higher overall incidence of burning sensation with tacrolimus ointment in adults.²⁴ Notably, this difference occurred upon initial

	Tacrolimus 0.03% ointment, n (%)	Tacrolimus 0.1% ointment, n (%)	Corticosteroid, ^b n (%)
Burning sensation			
Children (2–15 years)	35 (19) ^a	38 (20) ^a	13 (7)
Adults (≥16 years)	85 (45) ^a	113 (59) ^a	24 (13)
Pruritus			
Children (2–15 years)	25 (13)	21 (11)	14 (8)
Adults (≥16 years)	39 (20) ^a	29 (15) ^a	18 (10)
Erythema			
Children (2–15 years)	4 (2)	1 (<1)	3 (2)
Adults (≥16 years)	4 (2)	7 (4)	1 (<1)

^aP < 0.05 vs. corticosteroid. ^bHydrocortisone acetate and/or hydrocortisone butyrate ointments.

Table 3 Incidence of common application-site adverse events in 3-week double-blind studies of tacrolimus ointment vs. corticosteroid ointment in adults¹⁵ and children¹⁶

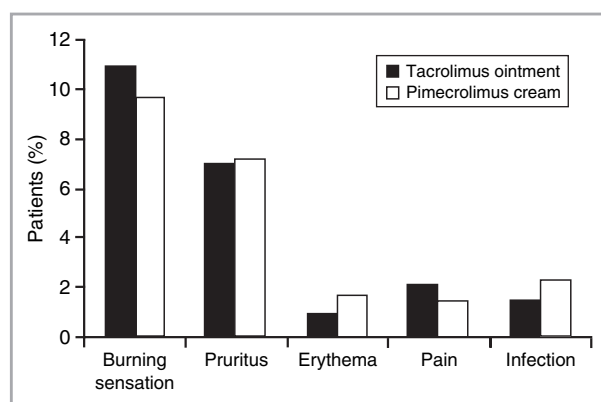


Fig 5. Percentage of patients with application-site adverse events in a combined analysis of three comparative studies of tacrolimus ointment vs. pimecrolimus cream.²⁴

treatment, and the incidence rates were similar by week 1 of the study in both treatment groups. In a fourth study, tacrolimus 0.03% ointment ($n = 70$) was compared with pimecrolimus 1% cream ($n = 71$) in a randomized, investigator-blinded, 6-week study in children (aged 2–17 years) with moderate AD.²⁵ The incidences of total AEs (84% for tacrolimus ointment vs. 86% for pimecrolimus cream) and application-site AEs (26% and 24%, respectively) were similar between the two groups. Local skin infections were uncommon, and there were no major differences between the two treatments. A greater percentage of patients receiving pimecrolimus cream discontinued as a result of AEs (7%) compared with those receiving tacrolimus ointment (1%).

Skin atrophy

As a result of their mode of action, topical corticosteroids inhibit the production of collagen and can cause dermal and epidermal skin thinning, resulting in skin atrophy.⁷¹ This process can start within 3 weeks of initiating therapy; indeed, subclinical skin damage may begin to occur within the first week.^{71–73} Treatment with a potent corticosteroid for only

3 days has been shown to result in a decrease in the rate of collagen synthesis that persisted for more than 2 weeks after cessation of treatment.⁷⁴ Unlike topical corticosteroids, tacrolimus ointment is not associated with skin atrophy. A randomized, double-blind, 7-day study in 12 healthy volunteers and 14 patients with AD showed that occlusive treatment with tacrolimus 0.1% or 0.3% ointment did not cause the adverse effects on collagen synthesis or skin thickness that were seen with betamethasone valerate 0.1% ointment.⁷⁵ In this study, the carboxy- and amino-terminal propeptides of procollagen I (PICP, PINP) and the amino-terminal propeptide of procollagen III (PIIINP) were measured from suction-blister fluid at the end of treatment. In addition, skin thickness was measured using ultrasound. The results for patients with AD and healthy subjects were similar. At the end of treatment, betamethasone-treated skin showed median PICP, PINP and PIIINP concentrations of 17.0%, 17.6% and 39.5% of the vehicle control, respectively, whereas the values for tacrolimus 0.1% and 0.3% ointments were approximately 100% of the vehicle control ($P < 0.001$).⁷⁵ In addition, median skin thickness was decreased by betamethasone by 7.4% relative to tacrolimus 0.1% ointment, 7.1% relative to tacrolimus 0.3% ointment and 8.8% relative to vehicle control ($P < 0.01$). Similar results were obtained in a study comparing pimecrolimus cream with betamethasone valerate and triamcinolone acetonide.⁷⁶

Not only is there no association between tacrolimus ointment and skin atrophy, but long-term treatment has been associated with improvements in collagen synthesis and skin thickness. In a 1-year open-label study, patients with moderate to severe AD received either tacrolimus 0.1% ointment ($n = 56$) or corticosteroids ($n = 36$), with 27 healthy volunteers acting as controls.⁷⁷ All the patients with AD had previously used topical corticosteroids and, at baseline, had decreased collagen synthesis rates relative to healthy individuals, as shown by concentrations of procollagen propeptides. Long-term tacrolimus ointment treatment was associated with an increase in collagen synthesis, with a median increase in combined procollagen propeptide concentrations of $272 \mu\text{g L}^{-1}$ (+140.9%, $P < 0.001$; Fig. 6), as well as a signifi-

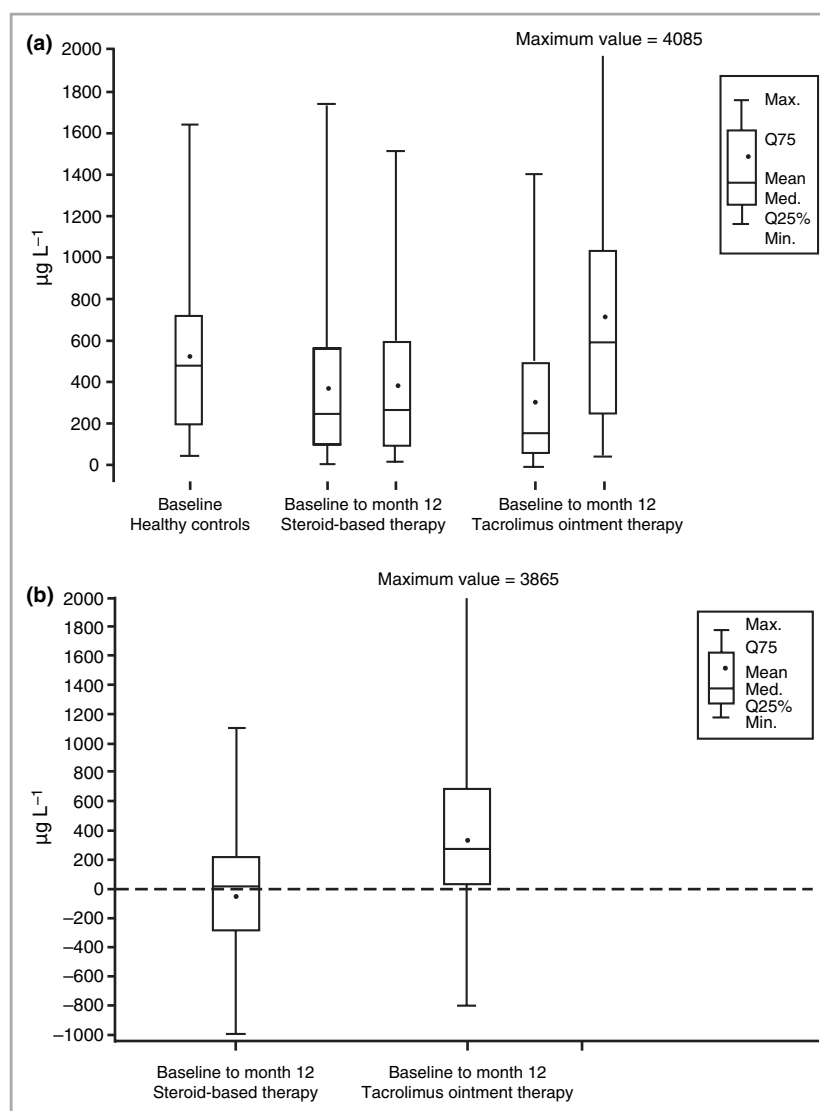


Fig 6. Procollagen propeptide I (PINP) and III (PIIINP) concentrations in patients with atopic dermatitis receiving tacrolimus ointment or topical corticosteroids and in healthy volunteers. (a) Sum of PINP and PIIINP values (absolute values). (b) Sum of PINP and PIIINP values (change from baseline to month 12).⁷⁶

cant increase in skin thickness (+9.0%, $P < 0.001$). Corticosteroid therapy did not significantly affect collagen synthesis beyond the reduced baseline levels that were observed in the patients with AD, but did produce a significant decrease in skin thickness (-8.2%, $P = 0.001$). In addition, skin atrophy progressively improved with long-term tacrolimus ointment treatment in three patients who had clearly visible signs of corticosteroid-related skin atrophy at baseline. Skin thickness and collagen synthesis increased and macroscopic signs of skin atrophy resolved.

Safety of tacrolimus ointment in the longer term

The safety of tacrolimus ointment in the longer term has been evaluated in three studies of 6–12 months' duration,^{13,14,18} and the safety of long-term intermittent tacrolimus ointment in adults and children has been assessed in three studies of up to 4 years' duration.^{19,20,22} Overall, the most common AEs

in these studies are application-site reactions and influenza-like symptoms (Table 4).

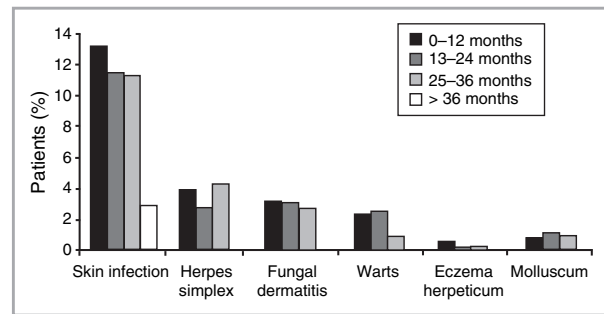
In two open-label, 12-month studies, treatment with tacrolimus 0.1% ointment was well tolerated in adults ($n = 316$) and children (aged 2–15 years; $n = 255$) with moderate to severe AD.^{13,14} In adults, the most common AEs were application-site reactions – burning sensation (43.7%), pruritus (19.0%) and erythema (8.9%) – that decreased in both incidence and intensity over time.¹³ The most common nonapplication-site AEs were influenza-like symptoms (21.8%; most frequently reported during the winter months), allergic reaction (20.9%), infection (14.2%) and headache (10.1%). Cox regression analyses demonstrated that there was no significant increase in the risk of skin infection or nonskin infection with increased cumulative use of tacrolimus ointment. Similar results were obtained in the paediatric study, with the most common application-site AEs being burning sensation (25.9%) and pruritus (23.1%).¹⁴ Again, these decreased in incidence over time. The most common nonapplication-site AEs were

Table 4 Incidence of common adverse events in long-term studies of tacrolimus ointment (≥ 12 months)^{13,14,18–20,22}

Adverse events (%)	Children (2–15 years)	Adults (≥ 16 years)
Application-site reactions		
Burning sensation	15.8–32.2	26.0–43.7
Pruritus	17.0–32.2	19.0–32.1
Skin infection	11.4–33.0	5.7–15.2
Influenza-like syndrome	28.2–47.0	21.3–30.1
Allergic reaction	8.4–26.2	5.5–13.0
Fever	7.9–24.9	1.7–4.4
Pharyngitis	9.9–22.7	4.0–5.1
Asthma	8.6–20.9	3.3–8.3
Headache	3.7–18.0	12.6–14.0
Increased cough	5.6–14.5	2.3–4.4

influenza-like symptoms (34.5%; apparently seasonal), headache (18.0%), fever (17.0%), asthma (16.1%) and allergic reaction (15.3%). The incidence of nonapplication-site AEs, including infections, did not increase with cumulative use of tacrolimus ointment or the duration of treatment, and most (85%) were considered to be unrelated to tacrolimus ointment. Overall, these two studies showed that the safety profile of tacrolimus ointment during up to 1 year of treatment was similar to that seen in short-term studies, with most AEs occurring at the start of treatment and being related to disease status.^{13,14} With the exception of influenza-like symptoms, which were considered to be seasonal in nature and unrelated to the use of tacrolimus ointment, there was no increase in the prevalence of any infection over time. The incidence of nonapplication-site events did not increase with prolonged use, and the incidence of cutaneous infections in both studies was low (11.1% in adults and 11.4% in children).^{13,14}

Although no 1-year comparative studies of tacrolimus ointment vs. corticosteroid treatment have been published, the results of the two 1-year studies discussed above are similar to those of a more recent 6-month study that compared tacrolimus 0.1% ointment ($n = 487$) with combined hydrocortisone butyrate 0.1% ointment and hydrocortisone acetate 1% ointment treatment ($n = 485$) in adults with AD.¹⁸ Both treatments were well tolerated; the most commonly reported AEs considered to be related to treatment were burning sensation (52.4% of patients receiving tacrolimus ointment, 13.8% of patients receiving corticosteroid ointment), pruritus (18.1%, 13.4%), folliculitis (11.7%, 10.1%) and alcohol intolerance (7.4%, 0.2%). Of these, only the incidences of burning sensations and alcohol intolerance (defined as application-site skin flushing after the consumption of alcohol) were significantly more common with tacrolimus ointment than with corticosteroids ($P < 0.001$). Although herpes simplex initially occurred more often in patients receiving tacrolimus ointment, the incidence decreased over time and, by month 6, it was similar in patients receiving tacrolimus ointment (1.3%) and corticosteroids (1.0%). All herpes simplex cases were mild to

**Fig 7.** Incidence of infections during up to 4 years of treatment with tacrolimus 0.1% or 0.03% ointment.²⁰

moderate in severity, apart from one patient in each treatment group who developed a severe infection. The so-called alcohol intolerance that was seen in some patients was generally mild to moderate in nature and did not lead to treatment discontinuation.

Further confirmation of the long-term safety of tacrolimus ointment has been demonstrated by three large-scale studies of up to 4 years' duration.^{19,20,22} The longest of these studies was a long-term, open-label, noncomparative study of tacrolimus 0.1% ointment in adults ($n = 408$) and children (aged 2–15 years, $n = 391$) in which patients were followed for up to 49 months, with 37.5% of patients being followed for longer than 3 years.²⁰ The most common application-site AEs over this time period were pruritus (26.3%), burning sensation (26.2%), skin infection (18.8%) and erythema (12.3%), and again the incidence was greatest at the start of treatment and decreased substantially over time. Most nonapplication-site AEs were unrelated to treatment and the most common were influenza-like symptoms (37.7%), allergic reaction (19.0%) and asthma (14.4%). The incidence of AEs, including skin infections, did not increase with time during the study, and the incidence of infections was low throughout (Fig. 7).

Overall, the results of these studies demonstrate that no new previously unreported AEs have emerged with long-term treatment. Tacrolimus ointment was well tolerated during long-term therapy, with a similar AE profile to that seen in short-term studies: the most common application-site AEs were burning sensation, pruritus and skin infection. These AEs occurred at the start of treatment and were related to disease status, decreasing in incidence over time as the AD improved. There was no increase in the incidence of any infection over time, with the exception of influenza-like symptoms, which were considered to be seasonal in nature. The incidence of nonapplication-site AEs did not increase with prolonged use of tacrolimus ointment and most of these events were unrelated to treatment.

Conclusions

In more than 14 years of clinical experience, the efficacy and safety of tacrolimus ointment for the treatment of AD have

been demonstrated in numerous short- and long-term studies. Systemic exposure to tacrolimus is minimal and transient after topical application, and the maximum blood concentration in most patients is below the limit of quantification. There is no evidence of a causal link between the use of tacrolimus ointment and the rare cases of skin cancer that have been reported. The safety profile of tacrolimus ointment, coupled with its demonstrated efficacy, makes it an important tool in the treatment of adults and children with AD.

Acknowledgments

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