SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion for the approval of Protopy. This scientific discussion has been updated until 1 October 2003. For information on changes after this date please refer to module 8B.

Introduction

The active substance of Protopy 0.03% ointment and Protopy 0.1% ointment is tacrolimus (FK506). One gram of Protopy 0.03% ointment contains 0.3 mg of tacrolimus (as monohydrate). One gram of Protopy 0.1% ointment contains 1.0 mg of tacrolimus (as monohydrate).

Tacrolimus is a macrolide immunosuppressant with which there are many years of clinical experience in the prevention and treatment of organ transplant rejection. A topical formulation has been developed for the treatment of atopic dermatitis.

Tacrolimus 0.03% and 0.1% ointment are indicated for the treatment of moderate to severe atopic dermatitis in adults who are not adequately responsive to or are intolerant of conventional therapies. Tacrolimus 0.03% ointment is also indicated for the treatment of moderate to severe atopic dermatitis in children who failed to respond adequately to conventional therapies.

In adults (16 years of age and above), treatment should be started with tacrolimus ointment 0.1% twice a day for up to three weeks. Afterwards the strength should be reduced to 0.03% twice a day. An attempt should be made to reduce the frequency of application if the clinical condition allows. Treatment should be continued until clearance of the lesion.

In children (2 years of age and above), only the 0.03% strength is indicated, treatment should be started twice a day for up to three weeks. Afterwards the frequency of application should be reduced to once a day until clearance of the lesion.

Atopic dermatitis (AD) is a chronic relapsing inflammatory skin disease, frequently seen in patients with a personal or family history of atopic diseases. Acute AD is characterised by intensely pruritic, erythematous papules that are associated with excoriations, erosions and serous exudates. Chronic AD is characterised by thickened skin with accentuated markings (lichenification) and fibrotic papules. During infancy, AD primarily involves the face, scalp and extensor surfaces of the extremities. In older patients, the flexural folds of the extremities are the predominant location for lesions.

Current management of AD involves hydration of the skin, the use of emollients and avoidance of skin irritants. Topical corticosteroids reduce inflammation and pruritus and are useful in controlling acute flares. Antihistamines are used to control pruritus. For patients with severe disease, resistant to topical corticosteroids, systemic corticosteroids, phototherapy (UVA and UVB), and systemic cyclosporin are utilised. Patients with AD have an increased susceptibility to a variety of microbial agents and topical or occasionally systemic antibiotics may be required to treat infection. Due to limited efficacy and adverse effects of current therapies, there is a need for a treatment for AD that is effective in severe disease, with a low potential for side effects with long-term use.

2. Part II: Chemical, pharmaceutical and biological aspects

The product contains tacrolimus in an oil-in-oil (o/o) ointment for the treatment of atopic dermatitis. The dossier was of good quality and no major queries arose from the initial assessment.

Composition

The ointment contains 0.03 % or 0.1 % of tacrolimus in an o/o ointment, which includes propylene carbonate and standard ointment excipients. Although propylene carbonate is not a common excipient, it is included in the US National Formulary and also in an FDA Inactive Ingredients Guide for topical preparations according to the Handbook of Pharmaceutical Excipients.

Active substance

Tacrolimus is not described in a pharmacopoeia. The specification proposed includes tests for identification, optical rotation, assay, related substances, residual solvents and general relevant pharmacopoeial purity tests. The related substances limits have either been justified with reference to

toxicological studies, and/or reduced. Tacrolimus is derived from the fermentation of *Streptomyces Tsukubaensis,* followed by extraction and purification of the active substance. Proof of structure has been demonstrated by the usual range of spectroscopic techniques.

Stability of the active substance

Three commercial-scale batches were stored in triple PE bags at 30°C for 24 months (one batch) and 36 months (two batches) and at 40°C/75 % RH for six months. Three pilot batches were stored in open Petri dishes at 30°C/75 % RH for three months and under 1000 lux light conditions for 50 days. The results show that active is stable under long-term and accelerated conditions and shows no instability to light and moisture. The re-test period of 24 months, with no storage restrictions, is acceptable.

Other ingredients

Excipients comply with BP, PhEur or US National Formulary monographs, as appropriate. Other than white beeswax which has no known TSE risk, no other ingredient of animal origin is used.

Product development and finished product

A topical product was developed as tacrolimus was expected to exert a local effect with reduced side effects. The development of the ointment included studies of solubility, pharmacological activity and dermal absorption with suitable solvents for the drug substance, and optimisation studies of ointment consistency (rheology) with various proportions of paraffins. The choice of excipients and quantities used have been adequately described and justified.

The 0.03 % ointment was tested and found to meet the PhEur. requirements for Antimicrobial Preservation, thus no preservative was added.

Manufacture of the ointment is carried out by Fujisawa Healthcare, in Grand Island, NY. The process consists of three stages: dissolution of tacrolimus, mixing of the resulting solution with a solution of base ingredients, and finally homogenisation of the ointment and cooling. Key steps have been optimised and the process was validated with data on nine commercial batches. Validation results show that homogenisation, storage and filling of the ointment does not have any adverse impact on the homogeneity of content throughout the tube and on droplet size.

The finished product specification includes appearance, assay, related substances tests, droplet size, as well as a test for consistency and homogeneity of the ointment. Related substances limits have been satisfactorily justified and/or amended.

Stability of the Product

Three production-scale batches of each strength in the 30 g and 60 g market pack size were stored for 36 months at 25°C/60 % RH, 12 months at 30°C/60 % RH and six months at 40°C/75 % RH. Three production-scale batches of each strength in the 10 g market pack size were stored for 12 months at 25°C/60 % RH and 12 months at 30°C/60 % RH. The stability study for the 10 g will be continued until completion at 36 months. A freeze-thaw study was also conducted. On storage, tacrolimus content declines over time and the levels of some related substances increase, but this is justified with reference to toxicological arguments. Based on available stability data, the proposed shelf-life and storage conditions as stated in the SPC are acceptable.

3. Part III: Toxico-pharmacological aspects

Pivotal studies were conducted under GLP guidelines.

Pharmacodynamics

Atopic dermatitis (AD) is a chronic, recurring inflammatory skin disease of uncertain aetiology. An increased number of antigen presenting dendritic cells, lymphocytes, macrophages, mast cells and eosinophils are found in lesional skin. The presence of activated T-helper cells, and the cytokines produced, appears to be important in both the initiation and chronic phases of AD.

In vitro studies on various cells (T cells, mast cells, basophils, eosinophils, Langerhans cells) and *in vivo* studies in animal models of allergic and spontaneous dermatitis were carried out to explore the pharmacodynamic effects of tacrolimus in atopic dermatitis (AD).

- *In vitro* studies

In vitro studies demonstrate a marked effect of tacrolimus on T helper cells, human peripheral blood eosinophils and human epidermal Langerhans cells. Cytokine production by T cells from human peripheral blood mononuclear cells and mouse spleen cells was about equally inhibited by tacrolimus and betamethasone. Langerhans cells, cultured in the presence of tacrolimus or betamethasone, reduced the stimulation of allogeneic T cells dose-dependently.

Via its binding to a specific cytoplasmic immunophilin (FKBP12), tacrolimus inhibits calcium-dependent signal transduction pathways in T cells, thereby preventing the transcription and synthesis of IL-2, IL-3, IL-4, IL-5 and other cytokines such as GM-CSF, TNF-α and IFN-γ.

- *In vivo* studies

Tacrolimus ointment (up to 1%) showed no effect on the early phase of the immediate hypersensitivity reaction in mice (passive cutaneous, ascaris antigen-induced peak ear oedema 1 hr after injection), but inhibited the late phase reaction (peak ear edema 24 h after injection); both reactions were inhibited by betamethasone ointment. Tacrolimus did not inhibit eosinophil or $CD4^+$ T cell infiltration in the late phase reaction, while betamethasone did. The delayed type hypersensitivity reactions in mice (tuberculin-induced and oxazolone-induced-contact delayed type hypersensitivity) were somewhat less affected by tacrolimus ointment than betamethasone ointment. In a mouse model of spontaneous dermatitis the effects of tacrolimus ointment inhibited the development of dermatitis, whilst steroid ointments had a marginal effect.
In general, in terms of the inhibition immune-mediated inflammation (i.e. delayed type hypersensitivity and late phase responses), tacrolimus ointment appeared to be somewhat less effective than glucocorticoid ointment.

- *Pharmacodynamic/kinetic drug interactions*

No topical interaction studies have been conducted with tacrolimus. Tacrolimus was practically not metabolised *in vitro* by human skin microsomes. Systemic exposure to topical tacrolimus is anticipated to be lower than that known with oral or intravenous exposure for which clinical experience outweighs pre-clinical experimental evidence.

- *General and safety pharmacology programme*

General pharmacological studies with tacrolimus were performed at doses up to 32 mg/kg orally or 3.2 mg/kg intravenously. Tacrolimus had no outstanding acute effects on the autonomic or motor nervous system, smooth muscle, kidney function or the haematological system; short-term continuous intravenous administration of tacrolimus affected the respiratory and cardiovascular system, and, albeit mildly, the central nervous and digestive system.
Systemic exposure to topical tacrolimus is anticipated to be low (see pharmacokinetics), therefore, acute systemic side effects are not anticipated with the use of tacrolimus ointment in the treatment of AD.

- Summary of salient findings

Tacrolimus is a macrolide immunosuppressant with which there are many years of clinical experience with oral and intravenous formulation for the prevention and treatment of organ transplant rejection. Pre-clinical studies have been conducted which demonstrate some pharmacological activity of tacrolimus in a topical formulation for the treatment of atopic dermatitis, but the detailed mechanism of action of tacrolimus in atopic dermatitis is not known. Because systemic exposure to topical tacrolimus is anticipated to be low, acute systemic side effects and drug interactions are not expected with the use of tacrolimus ointment in the treatment of AD.

Pharmacokinetics

Eight studies of tacrolimus ointment have been conducted to define the pharmacokinetics or toxicokinetics of tacrolimus in mouse, rat, rabbit, or micropig models. One *in vitro* study of the metabolism of ^{14}C-tacrolimus by rat skin microsomes was also conducted. In several of toxicological studies whole blood concentrations of tacrolimus were also determined as part of their evaluations. Tacrolimus concentrations were determined by enzyme immunoassay and liquid scintillation counting.

Topical administration pharmacokinetic studies suggest that absorption of tacrolimus is low, but highly variable. Tacrolimus does not penetrate beyond the epidermis to a significant extent in intact skin (absolute bioavailability: 4.7% in rat, 14.7% in rabbit, 0.94% in micro-pig). Penetration is higher in damaged skin (62.4% in the rat), as may be the case with AD lesions. In general, plasma levels increase with dose following oral and topical administration, nevertheless, there were some unexplained apparent changes in absorption with time which should not result in any adverse clinical impact. Systemically tacrolimus distributes widely and is almost completely metabolised by microsomal enzymes in the liver, metabolism in the skin is minimal. Excretion of tacrolimus is primarily via the bile and faeces. After systemic administration, tacrolimus crosses the placenta and is excreted into milk.

Toxicology

- **Single dose toxicity**

Tacrolimus ointment was applied to the shaved intact or abraded skin of rats at doses of up to 80 mg tacrolimus/kg, no death and no irritation or other dermal changes were seen.

- **Repeated dose toxicity**

Two dermal toxicity studies were conducted in the rat. In the 4 week study, 0-0(vehicle)-0.1-0.3-1% ointment (corresponding to 0-0(vehicle)-2-6-20 mg/kg tacrolimus) were applied daily to shaved, intact or abraded dorsal skin (10% body surface area (BSA)) for 6 hours a day. In the 26 week study, 0-0(vehicle)-0.03-0.1-0.3-0.5% ointment (corresponding to 0-0(vehicle)-0.6-2-6-10 mg/kg tacrolimus) were applied daily to shaved, intact dorsal skin (10% BSA) for 6 hours a day. Adverse effects were dose and time related. Mortality in the groups at 26 weeks was 7.5-12.5-10.0-0-22.5-27.5%, respectively. Principal adverse effects were: Skin – epithelial hyperplasia/acanthosis at the application site; Lymphoid – low white blood cell (WBC) and lymphocyte counts, thymus, spleen and lymph node atrophy; Renal – increased blood urea nitrogen (BUN), low K+, tubular basophilia, vacuolation and mineralisation; Blood – high RBC, low platelets; Pancreatic – islet cell vacuolation. After 26 weeks a dose related increase of lens opacities was noted (2.7-2.9-2.8-7.5-9.4-44.8%). The topical NOEL was calculated as 2 mg/kg/day tacrolimus for 4 weeks or 0.6 mg/kg/day tacrolimus for 26 weeks.

Two dermal toxicity studies were conducted in the Yucatan micro-pig. In the 13 week study, 0-0(vehicle)-0.1-0.3-1-3% ointment (corresponding to approximately 0-0(vehicle)-0.8-2.3-7.8-23.3 mg/kg tacrolimus) were applied twice daily to shaved, intact, unoccluded dorsal skin (40% BSA). In the 52 week study, an additional low dose group received 0.03% tacrolimus ointment twice daily (corresponding to approximately 0.2 mg/kg). There were no treatment related deaths. All treated animals had dermal papules, oedema, erythema, purple ring, hypopigmentation, multi-focal acanthosis, hyperkeratosis, mild chronic inflammation. Occasional increases in urinary K+ and decreased body weight gain in females at 3% ointment were also noted. Signs were in all treated groups, there was no dose relationship and a NOEL was not established. However, systemic exposure was lower in this species than in the rat.

In addition, data were provided from oral (13 weeks and 52 weeks) and intravenous (4 weeks) toxicity studies in rat and in baboon.

- **Reproduction studies**

A standard series of reproduction toxicity studies was conducted in rats and rabbits using the oral preparation. High doses were associated with: poor weight gain; reduced mating behaviour; prolonged diestrus; delayed parturition; increased pre-and post-implantation losses; reduced pup viability, failure to thrive (delayed testes descent); increased F1 variations and malformations (with relatively high incidence of ventricular septal defect). There was no effect on the developmental or mating parameters of those pups that survived to weaning. The maximum non-toxic dose levels were considered to be 0.32 mg/kg/day and 0.1 mg/kg/day tacrolimus in rats and rabbits respectively.

Although dermal absorption in humans is unlikely to produce the systemic levels of tacrolimus seen in these studies, the use of tacrolimus ointment in pregnancy or lactation is not recommended.

- **Genotoxic potential**

A standard series of genotoxicity studies, as per ICH guidelines, was conducted for tacrolimus. An Ames test and a mammalian chromosome aberration assay were conducted for the main degradation product. Overall, neither tacrolimus nor the degradation product caused genotoxic effects.

- **Carcinogenic potential**

Two carcinogenicity studies were conducted in mice. B6C3F1 mice, 50/sex/group, received 0-0(vehicle)-0.03-0.1-0.3-1-3% ointment (corresponding to approximately 0-0(vehicle)-1-3.3-10-33-100 mg/kg/day tacrolimus) for 104 weeks, applied topically to shaved intact skin over 40% of the total body surface area. The ointment remained on the skin for 24 hours and was wiped off prior to the next application. The animals were individually housed and were not collared during exposure, thus raising the possibility of oral ingestion. There was a dose related increase in mortality (17-15-22-59-85-100-100%), survival was insufficient for histopathological analysis in the top three doses. Principal lesions were treatment site inflammation and acanthosis, increased bone marrow myelopoiesis, cardiac degeneration and lymphoma. The incidence of these lesions followed the pattern of those found in the untreated mouse, but with increased frequency. An increased incidence of lymphoma was observed in animals treated with 0.1% tacrolimus. This is consistent with the immunosuppressive action of tacrolimus, most immunosuppressants are known to increase the incidence of lymphoma. In a second study, CD-1 BR mice received dietary dosing of up to 3 mg/kg tacrolimus. The pattern of lesions was similar to that observed in the dermal carcinogenicity study.
Two carcinogenicity (2 years) studies were conducted in rats by dietary administration (As the maximum tolerated dosage was not reached in the first study, a second carcinogenicity study was performed using higher dosages). The dose ranges used were 0-1.25 mg/kg/day and 0-5 mg/kg/day. The maximal non-toxic dose level in the first study was considered to 0.2 mg/kg/day tacrolimus. The pattern of toxicity mirrored that already discussed under repeated dose toxicity studies. There was no significant increase in neoplastic lesions compared to control. There was a slight fall in pancreatic islet cell and pituitary adenoma in treated rats.

Photo-toxicity

Studies were conducted to examine the effects of light on adverse event profile/genotoxicity of tacrolimus.
A standard bacterial reverse mutation assay was conducted in which half the plates in each treatment group were exposed to UVA/B of different intensities. An in vitro chromosome aberration assay was conducted in a similar manner. In neither case was there any evidence of genotoxicity in the absence or presence of UVA/B. In a photo-sensitisation assay, guinea pigs were exposed to topical tacrolimus and UVA for five consecutive days, followed three weeks later by a challenge test. Although moderate erythema was noted during the induction phase, there was no evidence of photo-sensitisation.
In a 13 week study, albino, hairless mice received 0-0(vehicle)-0.03-0.1-0.3-1.0-3.0% tacrolimus ointment (2 mg ointment/cm^2), applied topically to 40% of the body surface area, 5 days/week, and a dose of UV radiation from a solar simulator, alternately before or after application of ointment. Skin irritation, erythema, oedema etc., was seen in all treated groups including vehicle, but was dose and time related in severity. A subsequent 40-week study in albino, hairless mice used the same treatment regimen.
In a 52-week photocarcinogenicity study, albino hairless mice received 0-0(vehicle)-0.03-0.1-0.3-1% tacrolimus ointment (2 mg ointment/cm^2), applied topically to 20% of the body surface area, 5 days/week, and a dose of UV radiation from a solar simulator, alternately before and after application of ointment. The treatment was maintained for 40 weeks; thereafter the animals were maintained for a further 12 weeks without any treatment. The incidence of skin reaction was increased in all treated groups compared to sham control, in the absence of a dose relationship this appears to be a vehicle effect. The level of UV radiation used induces skin tumours in untreated mice, the highest dose group had significantly reduced onset time for tumours >1mm. Time to tumour was significantly reduced in treated males compared to vehicle, but not in females. The number of tumours >1mm per animal was increased in a dose related manner, vehicle compared to control and treated compared to vehicle (at 0.1% for males, 0.3% for females).

The underlying mechanisms may be a systemic immuno-suppression, however other mechanisms (local effect) cannot be excluded. In addition, photocarcinogenicity has also been observed with the vehicle for which the possible responsible component could be the liquid paraffin.

Nevertheless, the predictivity of the rodent photocarcinogenicity model (e.g. the albino hairless mouse model) for the human situation is at present unclear. Depending on the conclusions of the efficacy in humans, a warning to prevent excessive exposure to ultraviolet light in the SPC may be sufficient.

- **Local tolerance**

Single and repeated dose local tolerance studies were conducted in the rabbit. Some inflammation, with acanthosis and hyperkeratosis, was noted after several weeks dosing. Similar responses were seen in vehicle and treatment groups. The suitability of the vehicle was questioned. However, the reactions were not severe and tend to be self-limiting clinically. An additional sensitisation study conducted in the guinea pig showed no apparent reaction to tacrolimus.

- **Special toxicity studies**

Degradation Compound

The principle degradation compound of tacrolimus, found in the ointment, is called OD-1. The acute toxicity of OD-1 is low. Application of OD-1 (1% ointment) to intact rabbit or rat's skin, in single and repeated doses (4 weeks) respectively, produced no local or systemic effect. OD-1 is non-genotoxic *in vitro*. No additional health risk is anticipated from the presence of OD-1 in tacrolimus ointment.

- **Environmental risk assessment**

No environmental risk is anticipated from the use of tacrolimus ointment.

Discussion on toxico-pharmacological aspects

The minimum lethal dose in rats after a single application of tacrolimus ointment was >80 mg/kg. In 4- and 26-week dermal toxicity studies in rats the NOAEL was 0.1 and 0.03% respectively (2 and 0.6 mg/kg/day). No clear signs of systemic toxicity were seen in micro-pigs receiving tacrolimus at up to 3% topical (approximately 20 mg/kg/day), possibly due to the >10-fold lower systemic exposure in micro-pigs than in rats.
Systemic absorption of high doses tacrolimus is associated with a pattern of toxicity that includes lymphoid depletion, pancreatic, renal and lenticular changes, atrophy of male reproductive organs and adverse effects on sperm function. Immuno-suppression is the primary pharmacodynamic effect of tacrolimus, hence the lymphoid depletion. Diabetogenic-like symptoms have been noted with tacrolimus. It appears that the exposure levels associated with these effects greatly exceed those anticipated clinically with topical application of tacrolimus. Nonetheless these effects should be mentioned in the SPC (section 5.3 on preclinical safety data).

The proposed formulation of tacrolimus ointment is locally irritating. This may be due to the vehicle and an alternative was questioned, however the proposed formulation is acceptable because such effects are self-limiting in clinical use.

In a photocarcinogenicity study, tacrolimus shortened the latent period of UV-induced skin tumours beyond the vehicle effect. The underlying mechanisms for the photocarcinogenicity of tacrolimus ointment could be a systemic immuno-suppression, but a contribution from constituents of the ointment vehicle (e.g. liquid paraffin) could not be ruled out. Although the predictivity of this hairless mouse model is uncertain and the excipients are widely used in dermatological products, a risk of phototoxicity in clinical use cannot be excluded. Warnings should be included in the SPC to reduce the clinical risk (e.g. recommending reduced exposure to sunlight) and post-marketing monitoring of cutaneous malignancies should be planned.

A standard battery of genotoxicity studies was carried out with tacrolimus, which did not show genotoxic effects.

Reproduction toxicity studies were not performed with tacrolimus ointment. Reproductive and developmental toxicity as well as teratogenic effects were reported in oral reproduction toxicity studies. Although systemic absorption of tacrolimus from ointment is low, its use during pregnancy and lactation is not recommended.

4. Part IV: Clinical aspects

All studies are noted to have been performed to Good Clinical Practice.

The clinical development of tacrolimus ointment included within-subject patch tests in healthy volunteers, three pharmacodynamic studies in patients with AD, four phase II studies carried out to assess the optimal dose/concentration of tacrolimus (two in children and two in adults), five phase III comparative studies (three in adult patients and two in children) and one phase III comparative study comparing the efficacy of once daily and twice daily 0.03% tacrolimus in children. Data from long-term safety studies and Japanese studies were also provided.

Clinical pharmacology

The clinical pharmacology programme for tacrolimus ointment included:

- three studies with pharmacological parameters in AD patients (studies 97-0-030, FG-506-06-17 and FG-506-06-12).
- assessments of skin irritation, contact sensitisation, phototoxicity, photoallergy and photosensitisation in healthy subjects.

Pharmacodynamics

- Mechanism of action

A study (97-0-030) was performed using immunostaining of 16 cellular/cytokine markers in lesion biopsies of 21 AD patients treated with tacrolimus (0.1% ointment) or triamcinolone acetonide (0.1% ointment) twice daily for three weeks to target lesion. The pattern of changes in staining suggested that tacrolimus and triamcinolone may produce a similar clinical effect through different immunological mechanisms, but there were no definite conclusions on differences in the mechanism of action.

- Dynamic studies:

Tolerability in healthy subjects

Within-subject patch tests were conducted in healthy subjects to assess skin irritation, contact sensitisation, phototoxicity, photoallergy and photosensitisation.

Study	Treatment regimens	N
94-0-007 Single-centre, open, comparative to assess **Skin irritation**	Occlusive patches applied every 24 or 48 hours (weekends) for 3 weeks, containing: 0.03%, 0.1%, 0.3% tacrolimus/vehicle (ointment base)/0.005% calcipotriene/1.0% hydrocortisone/0.1% betamethasone valerate/0.5% sodium lauryl sulphate	30
94-0-004 Single-centre, open, comparative to assess **Contact sensitisation**	Occlusive patches applied every 48 (or 72 hours on weekends) for 3 weeks, containing: 0.03%, 0.1%, 0.3% tacrolimus/vehicle (ointment base)/0.005% calcipotriene/1.0% hydrocortisone/0.1% betamethasone valerate	30
95-0-011 Single-centre, double-blind, randomised to assess **Contact sensitisation**	Occlusive patches applied every 48 hours (or 72 hours on weekends) for 3 weeks containing: 0.03%, 0.1%, 0.3% tacrolimus/vehicle (ointment base)	229
94-0-005 Single-centre, open to assess **Phototoxicity**	All subjects received, in duplicate on 3 cm^2 treatment sites, a single application of: 0.03%, 0.1%, 0.3% tacrolimus/vehicle (ointment base) /0.005% calcipotriene/1.0% hydrocortisone/0.1% betamethasone valerate	12
94-0-006 Single-centre, open, to assess **Photosensitisation**	Occlusive patches applied 6 times over 3 weeks containing: 0.03%, 0.1%, 0.3% tacrolimus/vehicle (ointment base)/0.005% calcipotriene/1.0% hydrocortisone/0.1% betamethasone valerate	30

97-0-026 Single-centre, double-blind, randomised to assess **Photoallergy**	Occlusive patches applied 6 times over 3 weeks containing: 0.03%, 0.1% tacrolimus / vehicle (ointment base)	228

In the above-mentioned studies in healthy adults, tacrolimus ointment (0.03%, 0.1%, 0.3%) was shown to be mildly irritating, non-sensitising, but not associated with photo-toxicity, photo-allergy and photo-sensitisation.

Secondary pharmacology

Immunocompetence was assessed in a phase III, open-label, long-term study (FG-506-06-12) in a subset of AD patients. Recall antigen test and CD_4 and CD_8 counts did not change over time. However, recall antigen test is considered insensitive and only detects immunosuppression in a minority of patients with systemic immunosuppression. CD_4 and CD_8 counts only provide an estimate of T-cell function. The immuno-competence study was performed in adult patients and data from a paediatric population are lacking. Testing of immunocompetence should have utilised methods considered satisfactory for the investigation of primary immunodeficiency by the International Union of Immunological Societies (IUIS). In the absence of these specific data, the assessment of reports of infections is of major importance.

In contrast to betamethasone valerate, tacrolimus (0.1% and 0.3%) had no effect on collagen synthesis markers or skin thickness (surrogate markers for skin atrophy) in a short-term (7 days) study (FG-506-06-17) in 14 AD patients and 12 healthy volunteers. In preclinical models, reduction of skin thickness and skin atrophy were not observed.

Pharmacokinetics

- General:

The pharmacokinetic documentation for tacrolimus ointment consists of two *in vitro* studies, five pharmacokinetic studies involving 84 adult (≥ 16 years) and 28 paediatric (2-15 years) AD patients and data from 2015 patients enrolled in clinical efficacy and dose-finding studies.

Phase I and II studies in the US utilised a validated enzyme-linked immunosorbent assay (ELISA) which was modified to detect the low concentrations anticipated following topical treatment with tacrolimus. The limit of quantification (LQ) was 0.05 ng/mL and 0.5 ng/mL for the Phase III studies. In the European Phase II and III studies, a High Performance Liquid Chromatography with Mass Spectroscopy (HPLC-MS/MS) assay was utilised (LQ: 0.025 ng/mL). An ELISA was used in the Japanese studies (LQ: 0.05 ng/mL-0.5 ng/mL). In the systemic circulation, tacrolimus binds strongly to erythrocytes, resulting in a whole blood to plasma distribution ratio of approximately 20:1. Therefore, the pharmacokinetics of tacrolimus are based on measurements in whole blood.

In Vitro
The rate and the extent of penetration of tacrolimus (0.03-0.5%) through intact epidermis (human cadaver skin preparations) increased with increasing concentrations of ointment, and penetration was approximately seven-fold higher in skin sections devoid of stratum corneum than in intact skin.
A study using ^{14}C-tacrolimus and homogenates from two female cosmetic surgery donors did not show evidence of *in vitro* metabolism. The known hepatic M-I and M-II metabolites were not detected and recovery of radioactivity from the incubates was essentially complete.

Adult Healthy Volunteers
In healthy volunteers, minimal or no systemic absorption was observed following single or repeated application of 0.03%, 0.1% or 0.3% tacrolimus ointment to 1000 cm^2 intact skin. Of 14 subjects who participated, a distinct blood concentration profile (on Day 14 with 0.3% tacrolimus ointment) was found in only one subject (C_{max} 0.127 ng/ml, $t_{1/2}$ 68.6 hours, $AUC_{(0-24)}$ 2.31 ng.h/ml). It is noted that measurable levels of tacrolimus occurred only sporadically and the data was consistent with minimal absorption through intact skin.

Patients with Atopic Dermatitis
Patients with moderate to severe AD, ≥18 years old, received 0.1% tacrolimus ointment applied twice daily for 14 days. Pharmacokinetic assessments were carried out on Day 1, 4, 14 and every 24 hours for an additional 6 days.
Systemic exposure (AUC_{0-12}) increased from Day 1 to Day 4 and depended on the size of the treatment area. AUC_{0-12} values on Day 14 were similar to those on Day 1. At this time, lesions were essentially healed. In patients with highest BSA treated (> 6000 – 10000 cm^2), AUC_{0-12} on Day 1, Day 4 and Day 14 were 4.8±6.3, 10.2±9.2, 5.4±2.8 ng.h/ml respectively. The mean elimination half-life of 70 to 79 hours on Day 14 is longer than the half-life of intravenously administered tacrolimus (44 hours). Based on *in vitro* data and available *in vivo* data, the apparent long half-life following repeated administration is "absorption rate limited" (the rate and extent of penetration decreases as the skin heals leading to a lower rate of absorption compared with the rate of elimination). The long half-life reported in this study suggests that a longer dosing interval than the 12 hours used in most of the phase III clinical studies, could be appropriate.

In addition a study (FG01-506-09) to determine the distribution of tacrolimus in the skin of adult patients with moderate to severe atopic dermatitis following first and repeated application of 0.1% tacrolimus ointment is ongoing. Interim data (10 of 16 patients) demonstrate accumulation of tacrolimus in the skin following repeated administration and retention of measurable amounts in the skin in accordance with the long half-life in blood (70-80 hours).

In a European *Paediatric Pharmacokinetic Study*, patients with moderate to severe AD, 6-12 years old, received 0.1% tacrolimus ointment applied twice daily for 14 days to ≤1500 cm^2, >1500 to ≤3000 cm^2 or >3000 to ≤5000 cm^2 of body surface area. Pharmacokinetic assessments were carried out on Day 1 and 14. Systemic exposure was low (highest average systemic exposure observed: AUC_{0-12}= 8,4 ng.h/ml). Average exposure was highest in patients who treated about half of their total body surface area. It is noted that this strength is not recommended for use in paediatric patients and only the lower strength (0.03%) will be used.
A pharmacokinetic study was not carried out in children aged 5 years or younger, but some data were collected from the European paediatric phase III comparative study FG-506-06-19.

Blood concentrations determined in Clinical Studies
Tacrolimus whole blood concentrations were measured at scheduled visits in 12 clinical efficacy and dose-finding studies in which patients with AD received twice-daily application of 0.03%, 0.1% or 0.3% tacrolimus ointment. The duration of treatment was 3 weeks to 1 year. The distribution of patients by maximum tacrolimus blood concentrations show that 12.5% of paediatric patients and 27.0% of adult patients treated with 0.1% tacrolimus had tacrolimus concentrations ≥ 1 ng/ml. There was no evidence of systemic accumulation of tacrolimus in patients (adults and children) treated for prolonged periods (up to one year) with tacrolimus ointment.
In Phase III studies, 13.5% of patients (150/1109) with available data had concentrations ≥ 1 ng/mL and a subsequent measure showed a decrease of 20% in most patients indicating that systemic exposure is transient.

Discussion
Tacrolimus is absorbed following topical application. There is short-term accumulation of tacrolimus in the upper layers of skin prior to passive diffusion into the systemic circulation. In adult and paediatric pharmacokinetic studies systemic exposure was low but increased with increasing treatment areas. Most atopic dermatitis patients (adults and children) treated with single or repeated application of tacrolimus ointment (0.03 - 0.3%) had blood concentrations < 1.0 ng/ml. When observed, blood concentrations exceeding 1.0 ng/ml were transient. The absorption/penetration of topically applied tacrolimus decreases concurrently with lesion healing. There was no evidence of systemic accumulation following intermittent topical administration for periods of up to one year.
Tacrolimus blood concentrations normalised to the tacrolimus dose (0.03% or 0.1% ointment), were similar for 2 to 5 year olds compared with six to 15 year olds, indicating similar pharmacokinetics in both groups. In both adults and children with an average of 50% body surface area treated, systemic exposure (i.e. AUC) of tacrolimus from tacrolimus ointment is approximately 30-fold less than that

seen with oral immunosuppressive doses in kidney and liver transplant patients. The lowest tacrolimus blood concentration at which systemic effects can be observed is not known. However, 5 ng/ml can be considered an immuno-suppressive concentration as this concentration is used clinically in the latter phases of immuno-suppressive therapy in transplant patients. Approximately 19 % of atopic dermatitis patients in clinical studies experienced tacrolimus blood concentrations of $\geq$ 1 ng/mL and 1.3% of patients had a maximum concentration of ≥5 ng/mL. According to pharmacokinetic models, a Cmin in the 1 to 5 ng/mL range following topical application results in a systemic exposure corresponding to a Cmin of 0.5 to 2.5 ng/mL in transplant patients treated with oral tacrolimus. The applicant argued that this is unlikely to cause systemic immunosuppression as it is below the concentration range required for prevention of allograft rejection (5-15 ng/mL). Clinical safety data on tacrolimus ointment should be taken into consideration for assessing potential immunosuppressive effects.
Tacrolimus is extensively metabolised in the liver and although the concentrations are low following topical therapy, the product should be used with caution in patients with hepatic insufficiency (see section 4.4. of the SPC). Pharmacokinetics in special populations and gender effect were not studied.

- Interaction studies:

No formal interaction studies with tacrolimus ointment were performed. This was justified by the fact that an *in vitro* study showed that tacrolimus is not metabolised in human skin and systemic exposure with tacrolimus ointment will be minimal. Nevertheless, interaction with topical antimicrobial products for example cannot be excluded. Therefore the SPC should indicate that clinical infections at treatment sites should be cleared prior to commencing treatment with tacrolimus ointment and this should considered in post-marketing. Furthermore, there is a possible interaction with medications that are also metabolised by cytochrome P450 enzymes (e.g. CYP3A4). The concomitant administration of CYP3A4 inhibitors to patients with extensive disease including erythrodermic patients may result in significant interactions. This point should be addressed in the SPC with relevant examples CYP3A4 inhibitors provided.

Clinical efficacy

The tacrolimus ointment development programme included four phase II studies carried out to assess the optimal dose/concentration of tacrolimus (two in children and two in adults), six phase III comparative studies (three in adult patients and three in children). Data from the Japanese studies were also provided. All studies were conducted according to GCP.

All patients in the Phase II and III studies were diagnosed according to Hanifin and Rajka criteria of moderate to severe AD.

The Hanifin and Rajka Diagnostic Criteria for Atopic Dermatitis

Must have 3 or more major features:

- Pruritus
- Typical morphology and distribution: flexural lichenification of linearity in adults; facial and extensor involvement in infants and children
- Chronic or chronically relapsing dermatitis
- Personal or family history of atopy (e.g., asthma, allergic rhinitis, allergic conjunctivitis, atopic dermatitis)

Plus 3 or more minor features:

- Xerosis
- Ichthyosis/palmar
- Hyperlinearity/keratosis pilaris
- Immediate (type I) skin test reactivity
- Elevated serum IgE
- Early age of onset
- Tendency towards cutaneous infections
- Tendency towards nonspecific hand or foot dermatitis
- Nipple eczema
- Cheilitis

- Recurrent conjunctivitis
- Dennie-Morgan infraorbital fold
- Keratoconus
- Anterior subcapsular cataracts
- Orbital darkening
- Facial pallor/facial erythema
- Pityriasis alba
- Anterior neck folds
- Itch when sweating
- Intolerance to wool or lipid solvents
- Perifollicular accentuation
- Food intolerance
- Course influenced by environmental/emotional factors
- White, dermographism/ Delayed blanch

Dose-response studies and main clinical studies

Dose response studies

Four phase II studies were carried out to assess the optimal dose/concentration of tacrolimus, two with children and two with adults. Study FG-506-06-01 was conducted in Europe; the others were conducted in the United States. A total of 452 patients participated in these studies; 340 of whom received tacrolimus ointment. Criteria for success differed slightly between studies.

Overview of Study Design: Phase II Studies

	Protocol No. Patients	**Design**	**Treatment regimen**	**N (n*)**
Adults	**95-0-013** Age 17-69 years, ≥76% affected BSA	Multicentre, randomised, double-blind, sequential group, dose-escalation	Twice daily, 3 weeks, ≥76 BSA treatment area: • 0.03%, 0.1%, 0.3% tacrolimus • vehicle (ointment base)	26 (20)
	FG-506-06-01 Age 13-60 years, ≥200cm^2 affected BSA	Multicentre, randomised, double-blind, parallel group, comparative, dose-finding	Twice daily, 3 weeks, 200-1000 cm^2 affected skin: • 0.03%, 0.1%, 0.3% tacrolimus • vehicle (ointment base)	213 (159)
Children	**95-0-003** Age 6-16 years, 5-30% affected BSA	Multicentre, randomised, double-blind, parallel group, comparative, dose-finding	Twice daily, 3 weeks, maximum 20 g ointment/day: • 0.03%, 0.1%, 0.3% tacrolimus • vehicle (ointment base)	180 (136)
	95-0-009 Age 3-6 years, ≥25% affected BSA	Multicentre, randomised, double-blind, sequential group, dose-escalation	Twice daily, 3 weeks, treatment area of: • 25-50% or 51-75% BSA (0.03% tacrolimus or vehicle) • 51-75% or 76-100% BSA (0.1% tacrolimus or vehicle)	33 (25)

Population: all patients who received at least one application of study drug
BSA: body surface area ; N: total number of patients; n: number of patients who received tacrolimus ointment*

In the European study FG-506-06-01, patients applied vehicle (the ointment base, N=54) or 0.03% (N=54), 0.1% (N=54), or 0.3% (N=51) tacrolimus ointment twice daily to a selected area for 3 weeks. The primary endpoint was the change in treated area score 1 (the sum of ratings for erythema, edema, and pruritus, maximum=9). Treated area score 2 (maximum=21) combined score 1 with the sum of gradings (0-3) for oozing/crust, excoriation and lichenification, and dryness of noneczematous skin. A global assessment of response of the treated area was also performed (completely resolved, marked improvement, moderate improvement, slight improvement, no change, worse). Descriptive statistics for these endpoints is presented in the following table.

Main Efficacy Findings: Adult Phase II Dose-finding Study

	Vehicle (N=54)	Tacrolimus Ointment		
		0.03% (N=54)	0.1% (N=54)	0.3% (N=51)
Composite Scores for Treated Area–Median Percentage Decreases from Baseline to End-of-Treatment				
Score 1 (face and neck)	25.0	71.4	83.3	83.3
Score 1 (trunk and extremities)	22.5	66.7	83.3	75.0
Score 2 (face and neck)	27.3	70.6	75.0	77.8
Score 2 (trunk and extremities)	21.8	61.5	71.4	70.0
Investigator's Global Assessment of Treated Area – Number of patients (%)				
Completely resolved or markedly improved	5/53 (9.4)	31/53 (58.5)	43/53 (81.1)	36/51 (70.6)

ITT: all patients who were randomised, received at least one dose of treatment, and had at least one assessment for efficacy or safety after Day 1

Three times more patients discontinued treatment in the vehicle control group (21 patients, mostly due to prohibited therapy) than in the active treatment groups (7 in each treatment group). Treated area score 1 and score 2 showed greater improvement with 0.03%, 0.1% and 0.3% tacrolimus ointment compared with the vehicle control ($P<0.001$, Jonckheere test). Differences between active treatment groups were not statistically significant. The improvement was also greater for patients who received 0.03%, 0.1%, 0.3% tacrolimus compared with vehicle ($P<0.001$, Kruskal-Wallis).

The U.S. study 95-0-003 was conducted in paediatric AD patients. Patients applied vehicle (the ointment base, N=44) or 0.03% (N=43), 0.1% (N=49), or 0.3% (N=44) tacrolimus ointment twice daily for up to 3 weeks. Treatment areas were defined by the investigator at study entry and restricted to a maximum of 30% of the total body surface area. The primary endpoint was treatment success (≥75% improvement in the Physician's Global Evaluation of Clinical Response) at the end of treatment. The findings for this endpoint and secondary endpoints are summarised in the following table.

Main Efficacy Findings: Paediatric Phase II Dose-finding Study

	Vehicle (N=42)	Tacrolimus ointment		
		0.03% (N=42)	0.1% (N=46)	0.3% (N=43)
Patient Number (%)				
Treatment success [1]	16 (38.1)	29 (69.0)	31 (67.4)	30 (69.8)
Mean percent improvement from baseline to end of treatment				
Affected body surface area [2]	11.5	45.0	39.8	55.6
EASI [3]	25.7	71.6	76.5	81.1

Efficacy evaluable population: all patients with ≥5 applications during 3 consecutive days

[1] Difference between vehicle control and each tacrolimus concentration was significant ($P<0.05$) with no significant difference observed with pair-wise comparisons between the 3 tacrolimus ointment concentrations

[2] Difference among treatment groups was significant ($p<0.001$)

[3] EASI: Eczema Area and Severity Index (this early version of EASI does not correspond to the EASI used in the Phase III studies described below.

In summary, statistically significantly greater improvements with tacrolimus ointment as compared to vehicle were shown in the dose response studies. There were no statistically significant differences between the doses (although there was a suggestion of greater efficacy in 0.1% relative to 0.03% in the adult study) and it appears to be a dose-related incidence of application site adverse reactions. Thus the choice of 0.03% and 0.1% doses for further assessment was appropriate. A study investigating once versus twice-daily applications would have been appropriate, given the long systemic half-life following repeated administration. Such study was performed in children.

Main studies (phase III)

The programme included five twice daily Phase III comparative studies, three in adult patients and two in children. All had a multicentre, randomised, double-blind, parallel group design. A total of 2113 patients were included, 1414 of whom received 0.03% or 0.1% tacrolimus ointment. The two European trials (one in adults and one in children), which were designed to detect differences between treatment groups, used a reference therapy and so were limited to a three-week treatment duration. The three US trials (two in adults and one in children) were vehicle controlled with a twelve-week treatment duration.
In addition, a study was carried out to compare the efficacy of once daily and twice daily 0.03% tacrolimus in children (study FG-506-06-24).

Overview of Phase III Comparative Studies

	Protocol Patients	**Treatment regimen**	**N** **(n*)**
Adults	**FG-506-06-18** *Europe* Age 16-70 years, ≥5% affected BSA	Twice daily, 3 weeks, unrestricted treatment area: • 0.03%, 0.1% tacrolimus • 0.1% hydrocortisone butyrate	570 (384)
	97-0-035 *US* Age ≥16 years, ≥10% affected BSA	Twice daily, 12 weeks, unrestricted treatment area: • 0.03%, 0.1% tacrolimus • vehicle (ointment base)	304 (202)
	97-0-036 *US* Age ≥16 years, ≥10% affected BSA	Twice daily, 12 weeks, unrestricted treatment area: • 0.03%, 0.1% tacrolimus • vehicle (ointment base)	328 (218)
Children	**FG-506-06-19** *Europe* Age 2-15 years, 5-60% affected BSA	Twice daily, 3 weeks, treatment area #60% body surface area: • 0.03%, 0.1% tacrolimus • 1% hydrocortisone acetate	560 (375)
	97-0-037 US Age 2-15 years, ≥10% affected BSA	Twice daily, 12 weeks, unrestricted treatment area: • 0.03%, 0.1% tacrolimus • vehicle (ointment base)	351 (235)
	FG-506-06-24 *Europe* Age 2-15 years	Twice daily, 3 weeks: • 0.03% tacrolimus, • 1% hydrocortisone acetate, Once daily, 3 weeks: • 0.03% tacrolimus (with vehicle once daily for double-blinding purpose)	624 (417)

Population: all patients who received at least one application of study drug
BSA: body surface area
*N: total number of patients; *n: number of patients who received tacrolimus ointment*

The choice of hydrocortisone acetate 1% (mild topical corticosteroid) as comparator in the European paediatric trials was not considered optimal because of its known limited efficacy. Nevertheless, considering existing differences in prescribing practices within countries and the difficulty in proposing a corticosteroid that would be accepted in all cases, this choice was considered acceptable.

Exclusion criteria included clinically infected AD, infection requiring treatment, and clinically significant impairment of renal or hepatic function.

Demography
Patients had, on average, about one third of their bodies affected by AD lesions and about half had severe disease. There were no major differences in baseline characteristics across treatment groups.

Efficacy Endpoints
Efficacy parameters were the modified Eczema Area and Severity Index (mEASI), the physician's global evaluation of clinical response and physician's assessment of affected BSA.
The mEASI is a variant of the EASI, which has been validated, and which differs only in having an assessment of itch added. The mEASI itself has not been validated. It therefore includes individual ratings for erythema, oedema/induration/papulation, excoriations and lichenification as well as a score for the percentage of body surface affected. The mEASI area under the curve (AUC) in percent of baseline values was the primary endpoint in the European studies.
Physician's Global Evaluation of Clinical Response: Change from baseline was rated worse, less than 0%; no appreciable improvement 0 to 29%; slight improvements 30 to 49 %; moderate improvement 50 to 74%; marked improvement 75 to 89%; excellent improvement 90-99% and cleared to indicate 100% improvement. Treatment success was defined as cleared or excellent improvement and treatment success was the primary endpoint in the US studies but a secondary endpoint in the European studies.
Physician's Assessment of Affected Body Surface Area: Investigators estimated the percentage of BSA affected by AD (0-100%) for each of the four body regions (head and neck, trunk, upper limbs and lower limbs). Changes in affected BSA as a percent of total BSA was a secondary endpoint.

European Trials (3-week duration)

The mEASI mean area under the curve (AUC) as a percent of baseline was the primary endpoint in studies FG-506-06-18 and FG-506-06-19. This measurement considers the average mEASI from Day 1 to the end of treatment expressed as a percent of its baseline value.
Descriptive statistics for this endpoint are summarised in the following tables. Overall, the data show a dose-dependent therapeutic effect of tacrolimus in both adults and children.

Modified EASI as a Percent of Baseline – Mean Area under the Curve, European Trials
Intent-to-treat population: all randomised patients who received at least one application of study drug

	Reference Therapy*	Tacrolimus Ointment	
		0.03%	0.1%
Adults, FG-506-06-18	**N=183**	**N=187**	**N=186**
Mean±SD	47.7±44.5	52.3±28.5	41.7±24.9
Median (25% - 75%)	36.1 (24.7-60.7)	47.0 (32.4-68.1)	36.5 (23.4-53.4)
Children, FG-506-06-19, 2-15 years	**N=182**	**N=185**	**N=182**
Mean±SD	68.9 ± 33.2	50.5 ± 27.8	42.4 ± 20.0
Median (25% - 75%)	64.0 (45.0-84.7)	44.8 (31.8-62.5)	39.8 (29.5-53.9)
Children, 2-6 years	**N=94**	**N=91**	**N=91**
Mean±SD	68.6 ± 34.6	49.9 ± 27.9	39.7 ± 17.4
Median (25% - 75%)	63.7 (45.5-84.5)	41.8 (29.5-63.2)	38.2 (27.7-48.1)
Children, 7-15 years	**N=88**	**N=94**	**N=91**
Mean±SD	69.3 ± 31.9	51.0 ± 27.9	45.1 ± 22.1
Median (25% - 75%)	65.1 (45.0-84.8)	47.7 (35.7-62.1)	42.9 (30.3-58.1)

* 0.1% hydrocortisone butyrate ointment in adults; 1% hydrocortisone acetate ointment in children

Modified EASI as a Percent of Baseline, Mean Area under the Curve:
***P* values from Wilcoxon Rank Sum Test X, European Phase III Comparative Studies**
Intent-to-treat population: all randomised patients who received at least one application of study drug

	HyC-B[H] vs 0.1%	HyC-B[H] vs 0.03%[§]	HyC-A[I] vs 0.1%	HyC-A[I] vs 0.03%	0.1% vs 0.03%
Adults FG-06-506-18	0.343	0.002	-	-	<0.001
Children FG-506-06-19	-	-	<0.001	<0.001	0.006

[H] 0.1% hydrocortisone butyrate ointment [I] 1% hydrocortisone acetate ointment
[§] Greater improvement with 0.1% tacrolimus and 0.1% hydrocortisone butyrate than with 0.03% tacrolimus

In both the European adult and paediatric studies, the decrease in mEASI from baseline was evident by Day 4 of treatment for all treatment groups, with continued decreases over time in the tacrolimus ointment groups and hydrocortisone butyrate group

Finally, *study (FG-506-06-24)* in paediatric patients compared treatment with 0.03% tacrolimus once daily versus twice daily versus 1% hydrocortisone acetate twice daily for three weeks. The primary endpoint was the percentage change from baseline to the end of treatment in the modified Eczema Area and Severity Index (mEASI). The main efficacy findings are summarised in the table below.

MEASI, Percentage Decrease from Baseline (Day 1) to Week 3 / End of Treatment (ITT)

	Hydrocortisone Acetate 1%	Tacrolimus Ointment 0.03%	
		Once daily *	Twice daily
All patients	**N=207**	**N=207**	**N=210**
Mean ±SD	36.4±56.7	57.4±41.8	68.4±30.3
Median	47.2	70.0	78.7
Lower / upper confidence limits	42 / 57	65 / 76	76 / 83
Patients 2 to 6 years of age	**N=108**	**N=118**	**N=116**
Mean ± SD	29.5±64.0	60.7±40.0	70.6±28.3
Median	44.7	71.3	80.0
Lower / upper confidence limits	37 / 56	66 / 81	77 / 87
Patients 7 to 15 years of age	**N=99**	**N=88**	**N=94**
Mean ± SD	43.7±46.9	52.6±44.1	65.8±32.4
Median	53.7	67.4	76.6
Lower / upper confidence limits	39 / 68	54 / 77	68 / 82

*This population included one 1-year-old, who was excluded from the age-stratified analysis

In the overall population there was a difference in medians of 9 between once and twice daily 0.03% tacrolimus ointment. Statistically significantly better improvement was observed for twice-daily versus once-daily tacrolimus (p=0.007) and for either once daily or twice-daily tacrolimus versus twice-daily hydrocortisone acetate (p<0.001).

US-Trials (12 weeks duration)
"Treatment success" at the end of treatment (Week 12) was the primary endpoint and was defined as ≥90% improvement ("cleared" or "excellent") in the physician's global evaluation of clinical response.

Incidence of Treatment Success at the End of Treatment*

	Vehicle		Tacrolimus Ointment			
			0.03%		0.1%	
	N/N	%	N/N	%	n/N	%
Adults						
97-0-035	8/89	9.0	28/96	29.2	35/89	39.3
97-0-036	6/90	6.7	27/98	27.6	42/100	42.0
Children						
97-0-037						
All patients	8/101	7.9	39/108	36.1	44/107	41.1
2-6 years old	4/63	6.3	25/67	37.3	31/62	50.0
7-15 years old	4/38	10.5	14/41	34.1	13/45	28.9

*Efficacy evaluable population: all randomised patients with study drug for ≥3 consecutive days from baseline (≥5 applications) and ≥ 1 on-treatment value for the physician's global assessment.

Approximately three- to four-fold more patients in the tacrolimus treatment groups than in the vehicle group experienced treatment success (vehicle: 7-8%; 0.03%: ~35%; 0.1%: ~40%). The improvement in the tacrolimus-treatment groups compared with the vehicle-control groups was statistically significant for both adults and children. The greater improvement with 0.1% tacrolimus compared with 0.03% tacrolimus was significant only for the adult study 97-0-036.

Modified EASI as a Percent of Baseline (Baseline to Day 22), Mean Area under the Curve

	Vehicle		Tacrolimus Ointment			
			0.03%		0.1%	
	N	Median	N	Median	N	Median
Adults						
97-0-035	81	82.9	97	60.8	90	58.6
97-0-036	78	92.7	100	66.8	101	59.6
Children						
97-0-037	99	85.1	108	53.7	110	53.5

Modified intent-to-treat population: all randomised patients who received at least one application of study drug.

The mEASI mean AUC as a percent of baseline was about 25% higher in the vehicle control group than in the tacrolimus treatment groups ($P<0.001$, general linear models procedure).
The decrease in mEASI from baseline was evident by Week 1 of treatment in the two tacrolimus treatment groups with further decreases until the end of treatment.

Quality of life
All five phase III comparative studies showed improvements in quality of life as determined using validated questionnaires (Dermatology Life Quality Index in adult patients and the Children's Dermatological Life Quality Index in paediatric patients). In general, treatment differences paralleled the results for the efficacy endpoints.

Clinical studies in special populations

No studies in special populations other than children (from 2 to 15 years of age) were performed.

Exploratory analysis performed across trials (pooled analyses and meta-analysis).

To assess whether there were any prognostic factors that influenced the efficacy of 0.03% and 0.1% tacrolimus ointment in moderate to severe atopic dermatitis, the primary endpoint of each study was analysed by baseline disease severity, percent affected body surface area at baseline, and pre-baseline eczema treatments. Efficacy conclusions for all subgroups were the same as those for the overall population. There was no subgroup that showed a markedly different treatment response than the overall population.

Patients with high baseline lichenification scores (lichenified skin on at least three body regions) did not respond as well to treatment with tacrolimus as patients with low baseline scores. However, the response to tacrolimus was better than the response to vehicle. Treatment of lichenified lesions with tacrolimus seems appropriate.

To assess any influence of race on efficacy, stratified analyses by race of the primary endpoint of the European and U.S. phase III studies were carried out. The results suggest similar efficacy for different racial subgroups although it was noted that there was a greater treatment difference between the two doses in the black adult population.

Supportive studies

Long-term Studies

Two open-label, multicentre, long-term studies were conducted using only tacrolimus 0.1% ointment. (European study FG-506-06-12; ≥18 years, n=316; U.S. paediatric study 96-0-025; 2-15 years, n=255). Twelve-month data are available for 68 and 180 patients, respectively.

The efficacy results (mEASI and affected body surface area) confirm the results of the short term studies. Mean daily ointment use in adults during Months 3, 6, and 12 was about 60% of the mean daily usage during Week 1. This decrease in ointment use may have corresponded to decreases in the size of the treated area with the largest decreases occurring in the first week. Further clinical improvement was seen up to Month 3 and was maintained during the rest of the study. The long-term studies, however, were primarily done to assess safety.

Recurrence was assessed in the paediatric study. The average time to recurrence for these patients after the first AD episode was 55 days. Recurrence in the adult study was not monitored.

Japanese studies

In two Phase III comparative studies including a total of 329 patients 0.1% tacrolimus was found to have a similar efficacy to a potent topical corticosteroid (0.12% betamethasone valerate ointment) and a superior efficacy to a medium to low potency topical corticosteroid (0.1% alclometasone dipropionate ointment).

Discussion on clinical efficacy

The results of the pivotal efficacy studies indicate that tacrolimus is efficacious in the treatment of acute flares of moderate to severe AD and that the benefit is seen within a few days following commencement of treatment (according to secondary analysis). Nevertheless, pharmacokinetic, clinical and safety data as well as assessment of relapse/recurrence were taken into consideration to define the recommended posology.

Dose response

In the pivotal twice-daily studies, patients were not assigned to treatments according to severity of disease but were randomised to one of three treatment groups (vehicle/comparator, 0.03% or 0.1% ointment). Current management of AD involves the use of potent steroids for the treatment of acute flares and subsequent tapering with resolution of the condition. While the design of the pivotal studies does not reflect this clinical practice, it provides a useful opportunity to compare the efficacy and safety of the two strengths proposed for marketing. In the adult population, 12% more patients experienced treatment success with the higher dose and about a 10% greater improvement in the mEASI was seen with the higher concentration. It also seems that patients healed faster with the higher concentration. However, skin burning and alcohol intolerance also reached a higher incidence with the higher concentration. In addition, the systemic exposure increased with increasing concentration. In children the difference in response between the higher and the lower dose was less marked. These were taken into account in the recommended posology for each dose.

Half of the adults in the phase III comparative studies applied at least 3.9 to 5.6 g of tacrolimus ointment per day, and one quarter applied at least 7.4 to 10 g daily. Based on a pooled analysis of all seven phase III studies more than half of the patients treated more that 30% of their total body surface area. Approximately one third treated at least 50% of their body surface area. Increases in body

surface area treated were not associated with a change in benefit to risk balance and restrictions in ointment use do not seem necessary.

Twice-daily versus once-daily application
The twice daily dosing was questioned as kinetic data demonstrating a long half-life (initial half-life of 9 hours increasing to 70-80 hours following 14 days treatment) suggested that a longer dosing interval could be appropriate. Taking into account that efficacy has mainly been demonstrated with twice daily and that following repeated treatment there is a decrease in the rate (slower absorption than elimination) and extent of topical absorption of tacrolimus due to healing of skin lesions, a decrease in dosing frequency from twice daily to once daily may be possible after a clinical improvement in skin lesions, which should provide a better safety profile.

Intermittent short-term use versus continuous for a relapsing condition
In practice, patients with atopic dermatitis will most likely treat their disease when it is active and will modify the regimen as the condition heals either deliberately or because of poor compliance. Intermittent use is more appropriate than continuous use given the relapsing course of this condition. Since atopic dermatitis is a chronic disease treatment may be over long term. The studies performed support the use of tacrolimus as treatment of flares of moderate to severe atopic dermatitis 'as required', either short term or intermittent long term. This is reflected in the proposed posology.

Relapse/recurrence/rebound
The study designs included abrupt discontinuation of treatment and in some cases monitoring of recurrence. As the duration of treatment in the European and US pivotal studies were different (3 versus 12 weeks), the duration of treatment's impact on recurrence rate was questioned. As the disposition half-life increased to 80 hours after 14 days treatment, it was also questioned if this could impact on the incidence of relapse or recurrence. It was finally considered important to know whether tapering of dose could reduce relapse, recurrence or rebound.
Tapering of doses was not assessed in clinical studies. The design of the studies did not allow an appropriate retrospective analysis of relapse/recurrence/rebound rates, however, findings at follow-up for these studies did not suggest a large difference between the recurrence rate of patients treated for three or twelve weeks, nor suggested differences in rebound. It appears that recurrence occurred in approximately half of the patients two weeks after the end of treatment (US studies) and in the European studies half of the patients maintained moderate improvement at 2 week follow up.

Clinical safety

Patient exposure

Presentation of adverse events is made both by individual study and a pooled analysis of the five main pivotal trials. Because of the difference in duration of the European and US studies, the pooled analysis of incidence was limited to the first three weeks. To supplement the pooled analysis of incidence at week 3, Kaplan-Meier rates of adverse events at week 3 and week 12 were calculated for the 5 pivotal studies.

Adverse events and serious adverse events

Pivotal Studies

In the individual studies, the incidence of adverse events at the end of treatment was calculated for each treatment group, with treatment-differences assessed with Fisher's exact test. In the U.S. studies, statistical analysis of Kaplan-Meier rates of selected adverse events (using the generalised Wilcoxon test to assess treatment differences) was carried out because of the differences in time-on-treatment among the treatment groups (the U.S. studies had a very high drop-out rate in the vehicle control group, which led to a shorter duration of treatment for vehicle-treated patients).

European studies
The table below shows the incidence of most common adverse events, irrespective of causality.
In both adult and paediatric studies, *skin burning and pruritus* were the most common adverse events and were the only adverse events that were statistically significantly more common in the two tacrolimus

treatment groups. It was noted that over 90% of these adverse events were localised to the application site. It is also noted that these adverse events decreased in prevalence with time. Although skin burning occurred in up to 56.5% of patients in the adult studies in days 1-4, and it had reduced to 20.7% by days 5-8, a figure of up to 14.7% was still noted at week 2, and in the children, up to 19.9% day 1-4 had reduced to 4.9% at days 5-8.
Incidence of *infection adverse events* was analysed separately and folliculitis was the most common infection in adults (7-8%) and flu syndrome in children (around 8%). The incidence of infections was similar across treatment groups.

Incidence of most Common† Adverse Events, Irrespective of Causality: European Phase III Comparative Studies - Number of Patients (%)

Population: all randomised patients who received at least one application of study drug

	Reference Therapy ‡	Tacrolimus Ointment	
		0.03%	0.1%
Adults (FG-506-06-18)	**N = 186**	**N=193**	**N=191**
Skin & appendages			
Skin burning	24 (12.9)	87 (45.1)	113 (59.2)
Pruritus	18 (9.7)	39 (20.2)	30 (15.7)
Folliculitis	13 (7.0)	15 (7.8)	16 (8.4)
Body as a Whole			
Flu syndrome	12 (6.5)	8 (4.1)	12 (6.3)
Allergic reaction	12 (6.5)	6 (3.1)	5 (2.6)
Lack of drug effect	4 (2.2)	11 (5.7)	8 (4.2)
Nervous system			
Headache	14 (7.5)	10 (5.2)	9 (4.7)
Children (FG-506-06-19)	**N=185**	**N=189**	**N=186**
Skin & appendages			
Skin burning	13 (7.0)	35 (18.5)	38 (20.4)
Pruritus	15 (8.1)	25 (13.2)	22 (11.8)
Folliculitis	6 (3.2)	11 (5.8)	8 (4.3)
Body as a Whole			
Flu syndrome	16 (8.6)	15 (7.9)	14 (7.5)
Lack of drug effect	4 (2.2)	1 (0.5)	0
Nervous system			
Headache	3 (1.6)	1 (0.5)	3 (1.6)

† Common: experienced by at least 5% of patients in any treatment group in either study
‡ 0.1% hydrocortisone-17-butyrate in the adult study, 1% hydrocortisone acetate in the paediatric study.

In study (FG-506-06-24) comparing 0.03% tacrolimus ointment administered once and twice daily, and hydrocortisone acetate, safety data showed that application site adverse events were more frequent with twice daily dosing. (See table below). Both tacrolimus dosage regimens were associated with a higher level of skin burning and pruritus than hydrocortisone acetate.

Incidence of Common[H] Adverse Events, Irrespective of Causality-No. Patients (%)

Intent-to-treat population

Body System COSTART term	Hydrocortisone acetate 1%	Tacrolimus Ointment 0.03%	
		Once daily	Twice daily
	N=207	**N=207**	**N=210**
Application-site			
Body as a whole			
Lack of drug effect	10 (4.8%)	2 (1.0%)	6 (2.9%)
Skin and appendages			
Skin burning [I]	30 (14.5)	48 (23.2)	50 (23.8)
Pruritus	33 (15.9)	38 (18.4)	44 (21.0)
Folliculitis	8 (3.9)	8 (3.9)	11 (5.2)
Skin erythema	2 (1.0)	6 (2.9)	6 (2.9)
Rash	2 (1.0)	3 (1.4)	6 (2.9)
Skin infection	6 (2.9)	3 (1.4)	6 (2.9)
Pustular rash	5 (2.4)	3 (1.4)	3 (1.4)
Non-application-site			
Body as a whole			
Flu syndrome	11 (5.3)	6 (2.9)	12 (5.7)
Fever	4 (1.9)	5 (2.4)	6 (2.9)
Nervous system			
Headache	6 (2.9)	2 (1.0)	8 (3.8)
Respiratory system			
Pharyngitis	4 (1.9)	5 (2.4)	4 (1.9)
Cough increased	0	6 (2.9)	2 (1.0)
Asthma	1 (0.5)	5 (2.4)	2 (1.0)

[H] Common: at least 2% of patients in any treatment group

[I] $P \leq 0.05$; Cough increased, which showed an incidence of 0%, 2.9% and 1.0%, respectively, was the only other adverse event associated with a P value ≤ 0.05

US Studies

The table below shows the incidence of most common adverse events, irrespective of causality.

Skin irritation adverse events (skin burning, pruritus and skin erythema) were the most common adverse events in both the adult and paediatric studies and occurred more commonly in the tacrolimus groups than in the vehicle control groups. Over 90 % were localised to the application site. *Hyperaesthesia* occurred more commonly in the adult treatment groups.

Alcohol intolerance occurred in both adult tacrolimus treatment groups.

Flu syndrome and headache occurred more commonly in the adult tacrolimus groups although it is suggested that the higher incidence may be related to the lower incidence in the vehicle groups due to premature withdrawal due to lack of efficacy. In one of the three studies there is a suggestion that the incidence may be dose related.

It is noted that there was a higher incidence of flu syndrome, headache and otitis media in the paediatric tacrolimus treatment groups.

Adverse events showing a treatment effect that was consistent in both the analysis of incidence and the analysis of time-adjusted rates in at least one study were skin irritation adverse events (skin burning, pruritus and hyperesthesia/increased skin sensitivity), alcohol intolerance (facial flushing or facial skin irritation after consumption of alcohol), headache, allergic reaction (*e.g.*, allergic conjunctivitis, rhinitis, and allergic reaction to food), folliculitis and acne.

Unadjusted Incidence* of Most Common† Adverse Events, Irrespective of Causality: U.S. Adult Phase III Comparative Studies - Number of Patients (%)

Population: all randomised patient who received at least one application of study drug

Body System COSTAR T term	Study 97-0-035			Study 97-0-036		
	Vehicle	Tacrolimus Ointment		Vehicle	Tacrolimus Ointment	
		0.03%	0.1%		0.03%	0.1%
	N=102	**N=103**	**N=99**	**N=110**	**N=108**	**N=110**
Skin and Appendages	28 (27.5)	45 (43.7)	60 (60.6)	19 (17.3)	49 (45.4)	57 (51.8)
Skin burning	31 (30.4)	39 (37.9)	39 (39.4)	39 (35.5)	55 (50.9)	53 (48.2)
Pruritus	20 (19.6)	22 (21.4)	27 (27.3)	18 (16.4)	28 (25.9)	29 (26.4)
Skin erythema	6 (5.9)	8 (7.8)	2 (2.0)	10 (9.1)	14 (13.0)	6 (5.5)
Skin infection	5 (4.9)	3 (2.9)	1 (1.0)	6 (5.5)	2 (1.9)	5 (4.5)
Dry skin	1 (1.0)	2 (1.9)	3 (3.0)	3 (2.7)	3 (2.8)	8 (7.3)
Urticaria	3 (2.9)	3 (2.9)	6 (6.1)	2 (1.8)	4 (3.7)	9 (8.2)
Skin tingling	3 (2.9)	2 (1.9)	5 (5.1)	1 (0.9)	7 (6.5)	2 (1.8)
Herpes simplex	0	7 (6.8)	2 (2.0)	1 (0.9)	5 (4.6)	6 (5.5)
Folliculitis	2 (2.0)	4 (3.9)	4 (4.0)	0	4 (3.7)	8 (7.3)
Acne						
Body as a whole	9 (8.8)	20 (19.4)	19 (19.2)	13 (11.8)	21 (19.4)	33 (30.0)
Flu syndrome						
Allergic	6 (5.8)	11 (10.7)	5 (5.1)	2 (1.8)	10 (9.3)	4 (3.6)
Reaction	2 (2.0)	2 (1.9)	4 (4.0)	4 (3.6)	3 (2.8)	7 (6.4)
Accidental injury	0	2 (1.9)	4 (4.0)	0	4 (3.7)	8 (7.3)
Alcohol intolerance						
Nervous system	3 (2.9)	22 (21.4)	13 (13.1)	12 (10.9)	15 (13.9)	22 (20.0)
Headache	2 (2.0)	2 (1.9)	0	4 (3.6)	5 (4.6)	6 (5.5)
Insomnia	1 (1.0)	1 (1.0)	5 (5.1)	0	5 (4.6)	8 (7.3)
Hyperesthesia						
Respiratory system						
Asthma	2 (2.0)	7 (6.8)	2 (2.0)	1 (0.9)	3 (2.8)	5 (4.5)
Pharyngitis	1 (1.0)	6 (5.8)	2 (2.0)	3 (2.7)	0	5 (4.5)

* Due to a high drop-out rate in the vehicle groups, the average treatment period was approximately three-fold longer in the tacrolimus treatment groups than in the vehicle control groups.

† Common: experienced by at least 5% of patients in any treatment group in either study

Pooled Data from 5 Pivotal Studies (at 3 weeks)

The incidence of application site adverse events was higher in the tacrolimus or vehicle groups than in the corticosteroid groups and the tacrolimus group had an approximately 10% higher incidence of application site adverse events than the vehicle group. In all treatment groups these adverse events were considered to be causally related.

The incidence of non application site adverse events was similar across all treatment groups. Overall there were fewer adverse events noted in children.

Adverse events were analysed by age, gender, baseline severity, and by percent of BSA affected at baseline and none of the analyses showed a subpopulation at a greater risk of any given adverse

events. It was however noted that fever and cough occurred more often in children, and headache more often in adults; female patients tended to experience more skin burning, flu syndrome and headache and not surprisingly patients with severe disease and larger areas involved reported more skin burning, pruritus and skin erythema.

All application adverse events associated with tacrolimus were skin adverse events (skin burning, pruritus, skin erythema, hyperaesthesia). Approximately half of all patients treated with tacrolimus experienced skin irritation and the incidence was about 20% higher in adults. In both adults and children there was a higher incidence of skin irritation adverse events with the higher concentration of tacrolimus although this was significant only for skin burning in adults. The prevalence decreased over time. Common complications of AD such as skin infection, dry skin, pustular rash and face oedema appear to have been reduced in patients administered tacrolimus.

Alcohol intolerance (facial flushing/facial irritation after consumption of alcohol) occurred in 0.5% of the 0.03% tacrolimus group and 1.3% of the 0.1% tacrolimus group and in none of the patients who received vehicle or hydrocortisone butyrate.

In both adults and children, folliculitis had a greater incidence in tacrolimus or reference groups than in the vehicle groups. The incidence of skin infection was lower in patients treated with tacrolimus and reference compared with vehicle in both adults and children. The incidence of pustular rash in children was also reduced.
Herpes simplex occurred numerically (though not statistically significantly) more often in the tacrolimus groups and the rate was consistent with the prevalence observed in a patient population with AD.

Long-term Studies

The long-term (1-year) safety profile observed in the adult and paediatric long-term studies was similar to that observed in the short-term studies, with application-site irritation being a common adverse event at treatment onset.
In the adult study (FG-506-06-12), 5.1% of the patients experienced one or more serious adverse events. A causal relationship could not be excluded for herpes simplex (eczema herpeticum), herpes zoster (varicella), skin infection (staphylococcus superinfection), exacerbation of AD and cellulitis. In the paediatric study (96-0-025) 8.2% of the patients experienced one or more serious adverse events with skin infection, eczema herpeticum, and asthma as events for which a causal relationship could not be excluded.
In general, the long-term studies did not raise additional safety concerns.

Japanese Studies

In two Phase III comparative studies including a total of 329 patients, sensations of skin irritation at the application site (flush, tingling, itching) were reported in significantly more tacrolimus-treated patients than betamethasone-treated patients ($p<0.001$).
In a long-term safety study, in which patients with moderate to severe AD (n=568) applied 0.1% tacrolimus once or twice daily for 52 weeks showed similar overall findings to the European and U.S. long-term studies. The incidence of eczema herpeticum (5.6% of patients), however, was higher than observed in the U.S. and European studies. Overall, analysis of safety during a second year of treatment with 0.1% tacrolimus ointment was consistent with that from the first year of treatment.

Discontinuation due to adverse events

These adverse events occurred in 54/1414 (3.8%) and with a similar frequency in the vehicle and reference groups (42/699,6.0%).
In adults studies, skin burning and pruritus were the most common adverse events leading to discontinuation in the active treatment groups. The other reasons for discontinuation were: pruritus, skin infection, herpes simplex. In the US studies, more patients in the vehicle group had an adverse event leading to discontinuation than in the tacrolimus groups.
Discontinuations in the paediatric studies were due to skin infection, pruritus, skin burning/erythema, urticaria, pain (0.03%), asthma, 5 cases of chicken pox and one allergic reaction to food (0.1%).

Serious Adverse Events

Serious adverse events (SAE) occurred in 15/1414 (1.1%) and had a similar frequency in both vehicle and reference treatment groups (8/699,1.1%). SAEs in the tacrolimus groups were often related to the complications of atopic dermatitis such as skin infection, concomitant atopic infection disease such as asthma or allergic reactions, concomitant diseases present at baseline or common childhood or adult conditions.

Laboratory findings

Laboratory assessments included haematology, electrolytes, enzymes, glucose, creatinine and bilirubin. In all five pivotal studies, no consistent changes or notable differences among treatment groups in laboratory profile were observed.

Safety in special populations

Tacrolimus ointment has not been evaluated for its efficacy and safety in the treatment of clinically infected atopic dermatitis. Before commencing treatment with tacrolimus ointment, clinical infections at treatment sites should be cleared.

Tacrolimus ointment has not been evaluated in children below the age of 2 years.

A separate analysis was performed for elderly patients (>65 years) and showed a similar safety profile.

Discussion on clinical safety

According to pre-clinical data, a risk of photo-toxicity cannot be excluded. The potential for photocarcinogenicity is of concern, in particular as AD patients may be treated with UVB or PUVA. Phototoxicity has not been observed in a large clinical database. Therefore, patients have to be advised to minimise exposure of the skin to ultraviolet light, including sunlight and light from a solarium, and therapy with PUVA or UVB should be avoided.

Safety data demonstrate a pronounced irritating effect (application site adverse events considered causally related) of vehicle and at a higher level tacrolimus ointment. Although the vehicle is certainly not well tolerated, it is supposed to be the best pharmaceutical compromise. Such irritation effects tend to be self-limiting in clinical use.

The risk and aetiology of alcohol intolerance were considered. Vasodilatation was seldom in the five phase III comparative studies; 0.3% of patients who received vehicle and 0.3% and 0.6% of patients who received 0.03% and 0.1% tacrolimus ointment, respectively, reported this event. For almost all patients who reported this event, alcohol intolerance occurred at the application-site, which reflects local rather than systemic action. There was no association between alcohol intolerance and tacrolimus blood concentrations. Alcohol intolerance could be to the consequence of combined vasodilatative effects of alcohol and local irritating effects of tacrolimus ointment.

The main issue regarding safety of this product is the potential effect on Immunocompetence.

Pharmacokinetic data suggest the possibility of systemic exposure to tacrolimus with resulting potential immunosuppressant effect. The lowest tacrolimus blood level at which systemic effects can be observed is not known. In a retrospective analysis, which included adverse event data and tacrolimus blood concentrations from five phase III comparative studies, no conclusive relationship between systemic exposure and adverse events was discernible.

The techniques used to assess immunocompetence (Recall Antigen Test) are insensitive, and only detect immunosuppression in a minority of patients on systemic immunosuppression. Incomplete data presentation further compromises interpretation. The only available data are in adults, and cannot be extrapolated to children where immune system is under development. Therefore, tacrolimus ointment may potentially have a clinically relevant effect on cell-mediated immunity. Testing to evaluate immunocompetence should have utilised methods considered satisfactory for the investigation of primary immunodeficiency by the International Union of Immunological Societies (IUIS). In the absence of specific data, the assessment of the reports of infections is of major importance.

In each of the 5 pivotal studies the incidence of herpes simplex infection was higher in the 0.1% tacrolimus group as compared to the vehicle/reference therapy groups. In a pooled analysis of the data

of the five main phase III studies studies (12-week studies truncated at week 3), the differences observed were not statistically significant but an appreciable numerical difference between the control and tacrolimus groups was shown (see following table). Controlled long-term studies, which may help clarifying the issue, are not available. Therefore, the possibility that tacrolimus treatment leads to an increased incidence of herpes simplex infections cannot be ruled out. Accordingly, 'herpes simplex' was added to Section 4.8 of the SPC.

Incidence of Herpes Simplex Infection and Flu Syndrome, at Three Weeks from Baseline [% of patients] – Pooled Analysis of the Five Phase III Comparative Studies

Population: all randomised patients who received at least one application of study drug

	Vehicle	0.1% hydrocortisone butyrate	1% hydrocortisone acetate	Tacrolimus ointment		*P* value [H]
				0.03%	0.1%	
All patients	**N=328**	**N=186**	**N=185**	**N=710**	**N=704**	
Herpes simplex	0.6	0.5	0.5	1.8	2.1	n.s.
Flu syndrome	7.3	6.5	8.6	8.2	9.4	n.s.
Adult patients	**N=212**	**N=186**	**N=0**	**N=404**	**N=400**	
Herpes simplex	0.9	0.5	n.a.	2.7	2.8	n.s.
Flu syndrome	5.2	6.5	n.a.	7.4	9.0	n.s.
Children	**N=116**	**N=0**	**N=185**	**N=306**	**N=304**	
Herpes simplex	0.0	n.a.	0.5	0.7	1.3	n.s.
Flu syndrome	11.2	n.a.	8.6	9.2	9.9	n.s.

n.s.: not statistically significant ($P>0.05$); n.a.: not applicable

H Fisher's exact test for pair-wise comparisons of treatment groups

Regarding flu syndrome, data in the original documentation showed a dose-dependent substantial increase of flu syndrome in the 12-week US studies (but not in the 3-week studies). The analysis (see table above) by pooling the data of the five phase III comparative studies (12 week studies truncated at week 3) did not detect significant differences however, a Kaplan-Meier analysis at weeks 3 and 12 for Flu syndrome in US Phase III studies showed non statistical numerical differences, which does not rule out the possibility of an increased risk for flu syndrome. The data from non comparative long-term studies are not suitable for clarifying this issue. It is recognized, however, that the results from a total of 4116 patients in the follow-up study (99-0-054) do not establish that there is an increasing risk of flu syndrome (or herpes simplex) over time.

In addition, from the review of the Phase III clinical studies, folliculitis was identified as a treatment-related adverse event.

There were 33 cases of lymphadenopathy (0.08%) reported in clinical trials. The majority of these cases related to infections (skin, respiratory tract, tooth) and resolved with appropriate antibiotic therapy. However, as lymphadenopathy could be a marker of systemic immunosuppression, the SPC will specify that patients treated with tacrolimus ointment who develop lymphadenopathy should be monitored and investigated.

While monitoring of infection appears reassuring, if only a small percentage of patients experience significant systemic exposure, then larger studies are required to identify increased rates of infections in this subgroup of patients.

The effect of treatment with tacrolimus ointment on the immune system of children has not yet been established. The effect of tacrolimus ointment on the development of regulatory T cells has not been adequately investigated. Because of the ability of tacrolimus to inhibit the development of regulatory T cells, it is of concern that children, in particular, treated with tacrolimus may be more likely to become treatment dependent and less likely to 'grow out' of their disease.

5. Overall conclusions, benefit/risk assessment and recommendation

Quality

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

Preclinical pharmacology and toxicology

Tacrolimus is a macrolide immunosuppressant with which there are many years clinical experience in the prevention and treatment of organ transplant rejection. Overall, the primary pharmacodynamic studies provided some pharmacological activity of tacrolimus in a topical formulation for the treatment of atopic dermatitis but the exact mechanism of action of tacrolimus in atopic dermatitis is not known. From the pharmacokinetic point of view, topical administration studies suggested that absorption of tacrolimus is low and highly variable. Overall, the toxicology programme revealed that the proposed formulation is locally irritating and may have a potential for photocarcinogencity.

Efficacy

The results of the pivotal efficacy studies indicate that tacrolimus is effective in the treatment of acute flares of moderate to severe AD. In the adult population, the higher concentration showed a better efficacy. It also seems that patients healed with the higher concentration heal faster. In children the difference in response between the higher and the lower dose was less marked. The higher strength ointment is only proposed for use in the adults. Twice-daily application is more efficacious than once-daily application in the first two weeks. Following repeated treatment, there is a decrease in the rate (slower absorption than elimination) and extent of topical absorption of tacrolimus due to healing of skin lesions. Therefore, decrease in dosing frequency from twice daily to once daily may be possible after improvement in skin lesions, and should provide a better safety profile.

Safety

The most common adverse events were skin irritation adverse events (skin burning, pruritus and skin erythema). According to pre-clinical data, a risk of photo-toxicity cannot be excluded. Nevertheless, phototoxicity has not been observed in a large clinical database. The potential for photocarcinogenicity is of concern, in particular as AD patients may be treated with UVB or PUVA. Therefore, patients have to be advised to minimise exposure of the skin to ultraviolet light, including sunlight and light from a solarium, and therapy with PUVA or UVB should be avoided. The main safety issue is the potential effect on immunocompetence. Pharmacokinetic data show some degree of systemic exposure to tacrolimus with resultant potential immunosuppressant effect. The techniques used in this application to assess immunocompetence were insensitive and limited to adults. The incidences of herpes simplex infection and flu syndrome seemed higher, though not statistically significantly so, in the tacrolimus group than in the vehicle/reference therapy groups. Folliculitis was also identified as a treatment-related adverse event and lymphadenopathy may be a marker of systemic immunosuppression.

The effect of tacrolimus ointment on the development of regulatory T cells has not been adequately investigated. The effect of treatment with tacrolimus ointment on the developing immune system of children, especially the young, has not yet been established.

Benefit/risk assessment

The results of the pivotal efficacy studies indicate that tacrolimus is efficacious in the treatment of acute flares of moderate to severe AD. However, a concern remains regarding tacrolimus ointment potential for immunosuppression.

In view of the systemic absorption of tacrolimus, albeit minimal, and the potential for systemic immunosuppression, it is recommended that tacrolimus ointment should be reserved for the treatment of adult patients who are not adequately responsive to or are intolerant of conventional therapies (such as moderately potent topical steroids).
The effect of tacrolimus ointment on the development of regulatory T cells and on the immune system of children has not been established. It is therefore recommended that tacrolimus 0.03%, only, be reserved for the treatment of children (2 years of age and above) who failed to respond adequately to conventional therapies.
The treatment will also be restricted to dermatologists and physicians with extensive experience in the treatment of atopic dermatitis with immunomodulating therapy, because tacrolimus represents a new option (an immunomodulator) for a chronic disease to be used intermittently over long periods with the potential for immunosuppression.

As a consequence, tacrolimus ointment 0.1% for the treatment of adults and 0.03% for the treatment of adults and children can be approved for the following indications:
Tacrolimus 0.03% ointment
"Treatment of moderate to severe atopic dermatitis in adults who are not adequately responsive to or are intolerant of conventional therapies.
Treatment of moderate to severe atopic dermatitis in children who failed to respond adequately to conventional therapies."
Tacrolimus 0.1% ointment
"Treatment of moderate to severe atopic dermatitis in adults who are not adequately responsive to or are intolerant of conventional therapies."

Further studies to assess the impact of tacrolimus ointment on cell-mediated immunity should be performed and an intensive monitoring of infectious adverse events and malignancies (systemic and cutaneous) will be set up post-authorisation. Finally, further information on use in children (infants) below the age of 2 years is expected, as adverse atopic dermatitis is frequent in infants. The applicant has committed to a program of FollowUp Measures in order to resolve these issues within an agreed timeframe.

Recommendation

Based on the CPMP review of data on quality, safety and efficacy, the CPMP considered by consensus that the benefit/risk profile of Protopy (0.03% and 0.1%) in the treatment of moderate to severe atopic dermatitis in adults who are not adequately responsive to or are intolerant of conventional therapies was favourable as well as the benefit/risk profile of Protopy 0.03% in the treatment of moderate to severe atopic dermatitis in children (2 years of age and above) who failed to respond adequately to conventional therapies, and therefore recommended the granting of the marketing authorisation.