

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

The metabolism of pimecrolimus was investigated in the mouse, rat, rabbit, minipig and human using [³H] pimecrolimus under NDA 21-302. The parent compound was noted in blood as the main circulating component after oral or intravenous administration of [³H] pimecrolimus. In addition to the parent compound, many minor metabolites were noted in the blood of all tested species.

Metabolism in vivo was similar across all species and closely resembled metabolism in liver fractions in vitro. Metabolism involved a multitude of parallel and consecutive pathways. In addition, multiple slowly interconverting tautomers of the metabolites were observed. The predominant metabolic pathways in man were O-demethylations. The main pathway in human liver microsomes was O-demethylation at position 23 or 25, forming the metabolite designated as Dx_1 (P81.0). The two regioisomers could not be distinguished by LC/MS. Second and higher generation metabolites resulted mainly from combinations of the observed primary biotransformation reactions.

The results from this single oral dose pharmacokinetic study conducted in Cynomolgus monkeys suggested that the metabolism of pimecrolimus in Cynomolgus monkeys and in humans is similar. Therefore, it was determined that the Cynomolgus monkey would be a suitable non-rodent species for the 9 month oral toxicology study based on metabolism data.

No additional pharmacokinetic/toxicokinetic studies are recommended for pimecrolimus at this time.

Pharmacokinetic parameters were obtained in the single dose healthy volunteer clinical study and the 28 day clinical study in 1 patients. The AUC_{0-12 hr} value for the 60 mg/day dose (30 mg bid) was 281 ng/ml·hr after 28 days. The AUC_{0-24 hr} for the 60 mg/day dose would be 2X the AUC_{0-12 hr} value. Therefore the AUC_{0-24 hr} for the 60 mg/day dose would be ~562 ng/ml·hr (2 x 281 ng/ml·hr {day 28 value}). The 60 mg/day dose would correlate to the highest dose tested [



The highest AUC_(0-24 hr) value measured in humans after administration of Elidel cream under maximal use conditions was 38 ng·hr/ml and was measured in a single pediatric patient

that applied 1% pimecrolimus cream bid to 43.5% BSA. The multiple of human exposure levels based on nonclinical toxicity study data were calculated based on this $AUC_{(0-24 \text{ hr})}$ value, when nonclinical AUC data was available for comparison.

2.6.4.2 Methods of Analysis – HPLC with mass spectrometer analysis

2.6.4.3 Absorption - Refer to brief summary

2.6.4.4 Distribution - Refer to brief summary

2.6.4.5 Metabolism - Refer to brief summary

2.6.4.6 Excretion - Refer to brief summary

2.6.4.7 Pharmacokinetic drug interactions - Refer to clinical biopharmaceutics review

2.6.4.8 Other Pharmacokinetic Studies – N/A

2.6.4.9 Discussion and Conclusions – N/A

2.6.4.10 Tables and figures to include comparative TK summary – N/A

2.6.5 PHARMACOKINETICS TABULATED SUMMARY – N/A

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

Oral and dermal repeat dose toxicity studies were conducted in mice (duration up to 13 weeks), rats (duration up to 26 weeks) and minipigs (duration up to 26 weeks) and reviewed under NDA 21-302 to support Elidel cream. A brief tabular summary of toxicities after long term pimecrolimus treatment with associated NOAEL doses and corresponding AUC levels across species is provided in the following table.

Species	Duration/ Route	Dose (mg/kg/day)	Major findings
Mouse	13 weeks/ oral	0, 10, 50, 100, 312.5	NOAEL = 10 mg/kg/day; AUC _{0.5-4hr} = 1029 and 2949 ng·hg/ml in males and females, respectively ≥50 mg/kg/day: reduced lymphocyte count, cortical hyperplasia in thymic cortex, medullary thymic atrophy, pleomorphic lymphoid proliferation in spleen ≥100 mg/kg/day: reduced serum magnesium level, one lymphoma in spleen and mesenteric lymph nodes (female) 312.5 mg/kg/day: one lymphoma in thymus, islet cell vacuolation, uterine atrophy, vaginal epithelial hypertrophy (female)
Mouse (special studies)	13 weeks/ dermal (ethanol)	Study 1: 0.1 – 50 Study 2: 25 – 200	Study 1: NOAEL for lymphoproliferative changes = 10 mg/kg/day; AUC _{0-24hr} = 643 and 675 ng·hg/ml for males and females, respectively ≥ 25 mg/kg/day: lymphoproliferative changes, including malignancies Study 2: No NOAEL for lymphoproliferative changes was established in this study ≥ 25 mg/kg/day: lymphoproliferative changes, including malignancies ≥ 100 mg/kg/day: pleomorphic lymphoma noted after 8 weeks of treatment
Rats	26 weeks/ oral	0, 1, 5, 25	NOAEL = 1 mg/kg/day; AUC _{0-24 hr} = 17.5 and 23.7 ng·hr/ml for males and females, respectively ≥ 5 mg/kg/day: medullary thymic atrophy, reduced lymphocyte count, reduced serum magnesium level 25 mg/kg/day: reduced body weight, inflammatory lung lesions, islet cell vacuolation, reduced pancreas weight (female), tubular basophilia and mineralization of corticomedullary junction in kidney, thickening of urinary bladder, increased serum urea and urine volume, bone marrow atrophy, depletion of trabecular and cortical bone, lower pituitary and prostate weight, lens cataracts
Rats	26 weeks/ dermal (FMF)	0 (0%), 2 (0.2%), 6 (0.6%), 10 (1.0%)	NOAEL = 10 mg/kg/day (1% ASM 981 cream); AUC _{0-24 hr} = 4.9 ng·hr/ml for males and females Slight thickening of epidermis in control and 10 mg/kg/day groups. No systemic toxicity noted.
Minipigs (juvenile)	4 weeks/ oral	0, 5, 15, 45	NOAEL = 5 mg/kg/day; AUC _{0-24 hr} = 545 and 444 ng·hr/ml for males and females, respectively ≥ 15 mg/kg/day: lymphoid cell accumulation in

			adrenals 45 mg/kg/day: depression, diarrhea, reduced food consumption, decreased body weight, decreased serum phosphorus, calcium, magnesium and albumin, decreased thymus weight, thymus atrophy, minimal necrotizing arteritis (males)
Minipig	26 weeks/ oral	0, 2, 8, 30	NOAEL = 2 mg/kg/day; AUC _{0-24 hr} = 316 and 305 ng·hr/ml for males and females, respectively ≥ 8 mg/kg/day: accumulation of lymphocytes in adrenals, increased bronchi associated lymphoid tissue, decreased serum phosphorus and magnesium 30 mg/kg/day: mortality due to arteritis, decreased body weight gain, increased adrenal weight, decreased thymus weight
Minipig (juvenile)	13 weeks/ dermal (FMF)	0 (0%), 4 (0.2%), 12 (0.6%), 20 (1.0%)	NOAEL = 20 mg/kg/day (1% ASM 981 cream); no AUC could be determined in this study No systemic or local dermal toxicity was noted in this study.
Minipig	26 weeks/ dermal (FMF)	0 (0%), 4 (0.2%), 12 (0.6%), 20 (1.0%)	NOAEL = 20 mg/kg/day (1% ASM 981 cream); AUC _{0-24 hr} = 7.2 and 2.8 ng·hr/ml for males and females, respectively No systemic or local dermal toxicity was noted in this study.

The final study report for the 9 month oral monkey toxicology study was included in this submission and is reviewed in detail under the “Repeat-dose Toxicity” section below. A brief summary of the 9 month oral monkey toxicology study is provided below.

Oral (gavage) doses of 0, 15, 45 and 120 mg/kg/day pimecrolimus were administered to monkeys for 9 months. Treatment of high dose animals (120 mg/kg/day) was discontinued after 19 weeks of treatment due to severe clinical signs including mortality/moribundity. The first 2 surviving high dose animals/sex were treated as terminal sacrifices and necropsied in week 20 and the remaining 2 animals/sex were placed on a 20 week recovery period. The study was terminated after 39 weeks of dosing for the low (15 mg/kg/day) and mid dose groups (45 mg/kg/day) without additional recovery periods.

Treatment related lymphoid depletion/atrophy and increased macrophages in the thymus, decreased germinal center formation in the spleen and depletion of lymph nodes was noted in low, mid and high dose animals. Treatment related morphological changes included a spectrum of lymphoproliferative lesions that were diagnosed based on an adaptation of the diagnostic criteria for posttransplant lymphoproliferative disorders. Immunosuppressive related lymphoproliferative disorder (IRLD, equivalent to lymphoma) was noted in low (1/8; after 39 weeks of treatment), mid (5/8; after 7 {1 monkey} – 39 weeks of treatment) and high dose (7/9; after 14 – 19 weeks of treatment) animals. These treatment related changes were associated with a latent infection by lymphocryptovirus (LCV), which stained positive for EBER (Epstein Barr

related virus). No morphological evidence of LCV infection was noted in control animals or in high dose recovery animals. This indicates that the doses used in the monkey study demonstrated significant immunosuppression to allow expression of LCV in monkeys leading to IRLD. Other opportunistic infections in occasional animals included candidiasis of the oral mucosa (tongue, cheek pouch or soft palate) or esophagus and protozoiasis (amoebiasis and spironucleosis) of the large intestine. The presence of other opportunistic infections also indicates significant immunosuppression in monkeys after repeat dose treatment with pimecrolimus in this study.

IRLD noted in this study were associated with infection by LCV (an Epstein Barr related virus) and were similar to those described in immunosuppressed humans and monkeys following transplantation. Tissue distribution of IRLD varied by cases, but the most common extranodal (lymph node) sites were spleen, gastrointestinal tract, especially stomach and large intestine (often several levels being affected) and multiple segments of the respiratory tract (lung and nasal cavity). In human patients, post-transplantation lymphoproliferative disease (PTLD) is characterized by the potential of being fatal even at the early lesion stage. The possibility for remission of PTLD exists for most stages of the disease if the immunosuppressive therapy is discontinued at an earlier enough time. However, it has been noted in the transplant literature that the cases of PTLD that do not go into remission upon discontinuation of immunosuppressive therapy tend to be very aggressive and difficult to treat with chemotherapy. The possibility of reversibility after treatment with oral pimecrolimus in monkeys was suggested in this study by the lack of IRLD in high dose recovery animals and by the mostly negative hybridization for EBER in these animals. However, one of the recovery males had a small cluster of EBER hybridization in a retropharyngeal lymph node that morphologically exhibited mild reactive hyperplasia, but had no evidence of an IRLD.

A NOAEL for lymphoma (IRLD) was not established in this study.

During the review of the original NDA submission (NDA 21-302) for Elidel cream, the sponsor identified 5 byproduct impurities and 5 degradation products that were contained in the Elidel cream formulation. The sponsor had conducted adequate nonclinical toxicology studies to qualify all of the impurities and degradation products at the specified levels for Elidel cream.

The release limits for the degradation products that were qualified under NDA 21-302 were [

[The sponsor submitted a final study report to IND 54,217 (Serial Number 318; submitted 8-1-03) for a 4 week oral toxicity study in rats (impurity qualification: []
[These [] impurities were identified as degradation products. The structures for both degradation products are provided below. They are both modifications of the pimecrolimus structure.

The results of the 4-week oral rat toxicity study conducted to qualify the [] degradants suggest that a [] level of the [] degradant and a [] level of the [] degradant does not exaggerate the toxicity associated with pimecrolimus alone.

Genetic toxicology:

Pimecrolimus was negative in two in vitro bacterial mutagenesis assays (Ames test), an in vitro mammalian cell mutagenesis assay (L5178Y/TK+/- mouse lymphoma assay), an in vitro chromosomal aberration test in Chinese hamster cells and an in vivo mouse bone marrow micronucleus test.

The following information concerning pimecrolimus genotoxicity is included in the Elidel cream label.

A battery of in vitro genotoxicity tests, including Ames assay, mouse lymphoma L5178Y assay, and chromosome aberration test in V79 Chinese hamster cells and an in vivo mouse micronucleus test revealed no evidence for a mutagenic or clastogenic potential for the drug.

Appropriate genetic toxicology information has been incorporated into the Elidel cream label. No additional genetic toxicology studies are recommended for pimecrolimus at this time.

Carcinogenicity:

Several carcinogenicity studies (oral studies in mice and rats; dermal studies in mice and rats) have been conducted with pimecrolimus. A positive signal for malignant lymphoma was noted in the oral mouse carcinogenicity study and a positive signal for benign thymoma was noted in the oral rat carcinogenicity study.

In the 2-year rat dermal carcinogenicity study using Elidel cream, a statistically significant increase in the incidence of follicular cell adenoma of the thyroid was noted in low, mid and high dose male animals compared to vehicle and saline control male animals. Follicular cell adenoma of the thyroid was noted in the dermal rat carcinogenicity study at the lowest dose of 2 mg/kg/day (0.2% pimecrolimus cream).

No signal for either dermal or systemic carcinogenicity were noted in the dermal mouse carcinogenicity study. However, it has been established previously that a signal for malignant lymphoma was noted in a 13 week dermal toxicity study in mice that used much higher doses than were used in the dermal mouse carcinogenicity study. Therefore, it is possible to achieve lymphoma in mice after dermal application of pimecrolimus if the dose is high enough to allow sufficient systemic exposure to cause immunosuppression.

A 52 week dermal photo co-carcinogenicity study was conducted with Elidel cream in mice. A photo co-carcinogenic effect (decreased time to tumor formation) was noted for vehicle treatment in the photo co-carcinogenicity study in mice. No additional effect of Elidel cream treatment on tumor development beyond the vehicle effect was noted in this study.

The following information is included in the Elidel cream label for the carcinogenic potential of pimecrolimus.

In a 2-year rat dermal carcinogenicity study using Elidel Cream, a statistically significant increase in the incidence of follicular cell adenoma of the thyroid was noted in low, mid and high dose male animals compared to vehicle and saline control male animals. Follicular cell adenoma of the thyroid was noted in the dermal rat carcinogenicity study at the lowest dose of 2 mg/kg/day [0.2% pimecrolimus cream; 1.5X the Maximum Recommended Human Dose (MRHD) based on AUC comparisons]. No increase in the incidence of follicular cell adenoma of the thyroid was noted in the oral carcinogenicity study in male rats up to 10 mg/kg/day (66X MRHD based on AUC comparisons). However, oral studies may not reflect continuous exposure or the same metabolic profile as by the dermal route. In a mouse dermal carcinogenicity study using pimecrolimus in an ethanolic solution, no increase in incidence of neoplasms was observed in the skin or other organs up to the highest dose of 4 mg/kg/day (0.32% pimecrolimus in ethanol) 27X MRHD based on AUC comparisons. However, lymphoproliferative changes (including lymphoma) were noted in a 13 week repeat dose dermal toxicity study conducted in mice using pimecrolimus in an ethanolic solution at a dose of 25 mg/kg/day (47X MRHD based on AUC comparisons). No lymphoproliferative changes were noted in this study at a dose of 10 mg/kg/day (17X MRHD based on AUC comparison). However, the latency time to lymphoma formation was shortened to 8 weeks after dermal administration of pimecrolimus dissolved in ethanol at a dose of 100 mg/kg/day (179-217X MRHD based on AUC comparisons).

In a mouse oral (gavage) carcinogenicity study, a statistically significant increase in the incidence of lymphoma was noted in high dose male and female animals compared to vehicle control male and female animals. Lymphomas were noted in the oral mouse carcinogenicity at a dose of 45 mg/kg/day (258-340X MRHD based on AUC comparisons). No drug-related tumors were noted in the mouse oral carcinogenicity study at a dose of 15 mg/kg/day (60-133X MRHD based on AUC comparisons). In an oral (gavage) rat carcinogenicity study, a statistically significant increase in the incidence of benign thymoma was noted in 10 mg/kg/day pimecrolimus treated male and female animals compared to vehicle control treated male and female animals. In addition, a significant increase in the incidence of benign thymoma was noted in another oral (gavage) rat carcinogenicity study in 5 mg/kg/day pimecrolimus treated male animals compared to vehicle control treated male animals. No drug-related tumors were noted in the rat oral carcinogenicity study at a dose of 1 mg/kg/day male animals (1.1X MRHD based on AUC comparisons) and at a dose of 5 mg/kg/day for female animals (21X MRHD based on AUC comparisons).

In a 52 week dermal photo-carcinogenicity study, the median time to onset of skin tumor formation was decreased in hairless mice following chronic topical dosing with concurrent exposure to UV radiation (40 weeks of treatment followed by 12 weeks of observation) with the Elidel Cream vehicle alone. No additional effect on tumor development beyond the vehicle effect was noted with the addition of the active ingredient, pimecrolimus, to the vehicle cream.

No additional carcinogenicity studies are recommended for pimecrolimus at this time.

Reproductive toxicology:

The sponsor had previously conducted the following reproductive and developmental toxicology studies with pimecrolimus to support marketing of Elidel cream. These studies were reviewed under NDA 21-302.

- 1) Oral combined fertility and embryo-fetal development study in rats
- 2) Oral prenatal/postnatal embryotoxicity study in rats
- 3) Oral embryo-fetal development study in rabbits
- 4) Dermal embryofetal developmental study in rats
- 5) Dermal embryofetal developmental study in rabbits

The results of these studies were incorporated into the Elidel cream label. The following information is included in the Elidel cream label for the reproductive and developmental toxicology of pimecrolimus. Elidel cream is a pregnancy category C drug for the treatment of atopic dermatitis.

An oral fertility and embryofetal developmental study in rats revealed estrus cycle disturbances, post-implantation loss and reduction in litter size at the 45 mg/kg/day dose (38 × MRHD based on AUC comparisons). No effect on fertility in female rats was noted at 10 mg/kg/day (12 × MRHD based on AUC comparisons). No effect on fertility in male rats was noted at 45

mg/kg/day ($23 \times$ MRHD based on AUC comparisons), which was the highest dose tested in this study.

In dermal embryofetal developmental studies, no maternal or fetal toxicity was observed up to the highest practicable doses tested, 10 mg/kg/day (1% pimecrolimus cream) in rats ($0.14 \times$ MRHD based on body surface area) and 10 mg/kg/day (1% pimecrolimus cream) in rabbits ($0.65 \times$ MRHD based on AUC comparisons). The 1% pimecrolimus cream was administered topically for 6 hours/day during the period of organogenesis in rats and rabbits (gestational days 6-21 in rats and gestational days 6-20 in rabbits).

A combined oral fertility and embryofetal developmental study was conducted in rats and an oral embryofetal developmental study was conducted in rabbits. Pimecrolimus was administered during the period of organogenesis (2 weeks prior to mating until gestational day 16 in rats, gestational days 6-18 in rabbits) up to dose levels of 45 mg/kg/day in rats and 20 mg/kg/day in rabbits. In the absence of maternal toxicity, indicators of embryofetal toxicity (post-implantation loss and reduction in litter size) were noted at 45 mg/kg/day ($38 \times$ MRHD based on AUC comparisons) in the oral fertility and embryofetal developmental study conducted in rats. No malformations in the fetuses were noted at 45 mg/kg/day ($38 \times$ MRHD based on AUC comparisons) in this study. No maternal toxicity, embryotoxicity or teratogenicity were noted in the oral rabbit embryofetal developmental toxicity study at 20 mg/kg/day ($3.9 \times$ MRHD based on AUC comparisons), which was the highest dose tested in this study.

An oral peri- and post-natal developmental study was conducted in rats. Pimecrolimus was administered from gestational day 6 through lactational day 21 up to a dose level of 40 mg/kg/day. Only 2 of 22 females delivered live pups at the highest dose of 40 mg/kg/day. Postnatal survival, development of the F1 generation, their subsequent maturation and fertility were not affected at 10 mg/kg/day ($12 \times$ MRHD based on AUC comparisons), the highest dose evaluated in this study.

Pimecrolimus was transferred across the placenta in oral rat and rabbit embryofetal developmental studies.

The sponsor has repeated some of the reproductive and developmental toxicology studies conducted with pimecrolimus.

- 1) An oral fertility and early embryonic development study in rats
- 2) An oral embryo-fetal development study in rats
- 3) An oral embryo-fetal development study in rabbits

A summary of the results from these studies will be provided in this document. The sponsor conducted the reproductive and developmental toxicology studies in the rat with a different vehicle than the vehicle used for the studies submitted under NDA 21-302. The vehicle used in these studies provided for a much higher exposure in the high dose rat group compared to the previously conducted studies. It is anticipated that the sponsor repeated these studies with the different vehicle to allow for greater toxicity to be exhibited in the high dose group. Pimecrolimus demonstrated a signal for embryolethality in both rats and rabbits, but no signal

for teratogenicity. It is also recommended that the results from these studies be incorporated into an updated label for Elidel cream.

Special toxicology:

Elidel cream was a minimal irritant in the rabbit eye. Elidel cream did not elicit photoirritation in guinea pigs under UVA exposure conditions. This result is not surprising since the drug product does not absorb in the VIS/UVA range. It would have been preferable if this study utilized UVB exposure, since pimecrolimus showed absorption in the UVB range. Elidel cream did not induce phototoxicity in a human dermal safety study. Therefore, it was determined that repeat of the photoirritation study in guinea pigs with UVB exposure was not necessary.

No additional special toxicology studies are recommended for pimecrolimus at this time.

2.6.6.2 Single-dose toxicity

No nonclinical single-dose toxicity studies were included in this submission.

2.6.6.3 Repeat-dose toxicity

Repeat Dose Toxicity Study #1

Study title: 39-week oral (gavage) toxicity study in monkeys with a recovery period

Key study findings:

Treatment of high dose animals (120 mg/kg/day) was discontinued after 19 weeks of treatment due to severe clinical signs including mortality/moribundity. The first 2 surviving high dose animals/sex were treated as terminal sacrifices and necropsied in week 20 and the remaining 2 animals/sex were placed on a 20 week recovery period. The study was terminated after 39 weeks of dosing for the low (15 mg/kg/day) and mid dose groups (45 mg/kg/day) without additional recovery periods.

Treatment related mortality was noted at doses ≥ 45 mg/kg/day. Three mid dose animals were sacrificed moribund, one high dose animal was found dead and four additional high dose animals were sacrificed moribund. All moribundity/mortality was associated with an immunosuppressive related lymphoproliferative disorder (IRLD). IRLD is equivalent to lymphoma. A treatment related decrease in body weight was noted at doses ≥ 45 mg/kg/day.

Pimecrolimus treatment produced most of its effects on the hematopoietic system. Bone marrow smears from nine pimecrolimus treated monkeys (4 mid dose monkeys, 5 high dose monkeys) were diagnosed with IRLD based on the bone marrow analysis. Three of these monkeys had concurrent leukemia. Increased numbers of stimulated lymphocytes, immunoblasts, plasmacytoid cells and/or atypical lymphocytes, most prominently monocytoid forms were commonly identified in the pimecrolimus treated monkeys. These observations were

independent of concurrent lymphoproliferative disease. Seven of nine monkeys with bone marrow disease experienced erythrocyte depression ranging from minimal to blatant anemia. Increased aPTT (coagulation abnormality) was noted in mid and high dose animals.

Treatment related lymphoid depletion/atrophy and increased macrophages in the thymus, decreased germinal center formation in the spleen and depletion of lymph nodes was noted in low, mid and high dose animals. Treatment related morphological changes included a spectrum of lymphoproliferative lesions that were diagnosed based on an adaptation of the diagnostic criteria for posttransplant lymphoproliferative disorders. Immunosuppressive related lymphoproliferative disorder (IRLD, equivalent to lymphoma) was noted in low (1/8; after 39 weeks of treatment), mid (5/8; after 7 {1 monkey} – 39 weeks of treatment) and high dose (7/9; after 14 – 19 weeks of treatment) animals. These treatment related changes were associated with a latent infection by lymphocryptovirus (LCV), which stained positive for EBER (Epstein Barr related virus). No morphological evidence of LCV infection was noted in control animals or in high dose recovery animals. This indicates that the doses used in the monkey study demonstrated significant immunosuppression to allow expression of LCV in monkeys leading to IRLD. Other opportunistic infections in occasional animals included candidiasis of the oral mucosa (tongue, cheek pouch or soft palate) or esophagus and protozoiasis (amoebiasis and spironucleosis) of the large intestine. The presence of other opportunistic infections also indicates significant immunosuppression in monkeys after repeat dose treatment with pimecrolimus in this study.

IRLD noted in this study were associated with infection by LCV (an Epstein Barr related virus) and were similar to those described in immunosuppressed humans and monkeys following transplantation. Tissue distribution of IRLD varied by cases, but the most common extranodal (lymph node) sites were spleen, gastrointestinal tract, especially stomach and large intestine (often several levels being affected) and multiple segments of the respiratory tract (lung and nasal cavity). In human patients, post-transplantation lymphoproliferative disease (PTLD) is characterized by the potential of being fatal even at the early lesion stage. The possibility for remission of PTLD exists for most stages of the disease if the immunosuppressive therapy is discontinued at an earlier enough time. However, it has been noted in the transplant literature that the cases of PTLD that do not go into remission upon discontinuation of immunosuppressive therapy tend to be very aggressive and difficult to treat with chemotherapy. The possibility of reversibility after treatment with oral pimecrolimus in monkeys was suggested in this study by the lack of IRLD in high dose recovery animals and by the mostly negative hybridization for EBER in these animals. However, one of the recovery males had a small cluster of EBER hybridization in a retropharyngeal lymph node that morphologically exhibited mild reactive hyperplasia, but had no evidence of an IRLD.

A NOAEL for lymphoma (IRLD) was not established in this study.

Study no.:	0370001
Volume #, and page #:	1, 1
Conducting laboratory:	Novartis Pharmaceutical Corporation, East Hanover, New Jersey
Date of study initiation:	2-13-03
GLP compliance:	Yes

QA report: Yes
Drug, lot #, and % purity: ASM 981 solid solution, batch# X065 0402
Vehicle: Aqueous solution containing 0.5% Poloxamer 188 and 3.5% hydroxypropylmethyl cellulose (Note: The stock oral ASM 981 solution was referred to as ASM 981 solid solution and consisted of 14% ASM 981 (w/w) solid dispersion in 0.5% Poloxamer 188 and 3.5% hydroxypropylmethyl cellulose as a carrier in water.)

Methods

Doses: 0, 15, 45 and 120 mg/kg/day
Species/strain: Cynomolgus monkey
Number/sex/group or time point (main study): 4/sex/dose
Route, formulation, volume, and infusion rate: oral (gavage), solution, 10 ml/kg, bolus
Satellite groups used for toxicokinetics or recovery: 2/sex/dose for recovery in control and high dose groups
Age: 2.5 – 3.5 years
Weight: males: 2.2-4.0 kg; females: 2.1-3.4 kg
Sampling times: described in following section
Unique study design or methodology:

Test article or vehicle (aqueous solution containing 0.5% Poloxamer 188 and 3.5% hydroxypropylmethyl cellulose) was administered orally (via gavage) on a daily basis, 7 days/week, for 39 weeks. Due to severe clinical signs including moribundity, treatment of high dose animals was discontinued after 19 weeks of treatment and the last 2 surviving animals were placed on a 20 week recovery period. The study was terminated after 39 weeks of treatment (including control animals) without additional recovery periods.

Observation and Times:

Clinical signs: daily
Body weights: weekly
Food consumption: daily
Ophthalmoscopy: prior to exposure and for all surviving animals during week 39 (or recovery week 20 for high dose animals)
EKG: prior to exposure and for all surviving animals ~1.5-2 hours after administration during week 39 (or recovery week 20 for high dose animals)
Hematology: prior to exposure and in weeks 6, 13, 19 (high dose only), 25, 32 and 39
Clinical chemistry: prior to exposure and in weeks 6, 13, 19 (high dose only) and 39
Plasma was collected predose and at the end of treatment for determination of estradiol and testosterone concentrations.
Urinalysis: prior to exposure and in weeks 6, 13, 19 (high dose only) and 39
Gross pathology: necropsy

Organ weights: adrenal, brain, heart, kidney, liver, ovary, pituitary, prostate, spleen, testes, thyroid, uterus

Histopathology: The following organs were preserved from all animals in all treatment groups: adrenals, aorta, bone with bone marrow (femur, sternum), brain, epididymis, esophagus, eyes, gallbladder, heart, large intestines (colon, cecum, rectum), small intestines (duodenum, jejunum, ileum), kidneys, lacrimal gland, liver, lungs, lymph nodes (bronchial, mandibular, mesenteric), mammary gland, ovaries, pancreas, pituitary, prostate, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid/parathyroid, tongue, trachea, uterus with cervix, urinary bladder, vagina and all gross lesions.

Histological examination was performed on all listed organs for all dose groups. In addition to the traditional H & E staining, special stains were used for histopathological analysis in this study. Immunohistochemical methods were used to detect the following: CD3, CD15, CD20, CD30, CD68, λ , κ , EBNA-2, Ki-67, BZLF1, sVCA-p18. In situ hybridization was utilized to detect EBER-1.

Toxicokinetics: Blood samples for toxicokinetic analysis were obtained from nonrecovery animals on days 1/2 and during weeks 4, 19 (high dose only) and 38/39. Blood samples were collected at 1, 2, 4, 8 and 24 hours post dose. Plasma levels of pimecrolimus were determined by HPLC/MS.

Results:

Note: All treatment related changes are versus control unless otherwise stated.

Mortality: Treatment related mortality was noted at doses ≥ 45 mg/kg/day. Three mid dose animals were sacrificed moribund on days 48, 226 and 233. One high dose animal was found dead on day 103 and four additional high dose animals were sacrificed moribund on days 110, 125, 127 and recovery day 6. All moribundity/mortality was associated with an immunosuppressive related lymphoproliferative disorder.

Clinical signs: Clinical observations noted in animals prior to moribund sacrifice/death at doses ≥ 45 mg/kg/day included pale appearance, cold to touch, decreased locomotor activity, ataxia, muscle tremors, constricted pupils, labored or shallow respiration, gasping, rales, pale gums, abdominal distention, lacrimation and/or clear nasal discharge.

Treatment related fecal changes (soft feces and/or diarrhea) were noted at doses ≥ 15 mg/kg/day and emesis was noted at doses ≥ 45 mg/kg/day. Lymph node swellings were noted in high dose animals. Visible lymph node swellings were no longer apparent in high dose animals by recovery day 22.

Body weights: A treatment related decrease in mean body weight was noted in mid dose animals (males: ↓9.3%; females: ↓9.1%) by day 273 and high dose animals (males: ↓5.9%; females: ↓10.7%) by day 127 (end of dosing for high dose group).

A treatment related decrease in mean body weight gain was noted in low dose animals (males: ↓20.0%; females: ↓33.3%) by day 273, mid dose animals (males: ↓26.7%; females: ↓44.4%) by day 273 and high dose animals (males: ↓66.7%; females: ↓66.7%) by day 127 (end of dosing for high dose group).

Body weight changes were reversible in the high dose group during the recovery period.

Food consumption: A minor treatment related decrease in food consumption was noted in high dose males and mid and high dose females. Food consumption returned to normal during the recovery period.

Ophthalmoscopy: No treatment related effects on ophthalmic parameters were noted in this study.

EKG: No treatment related effects on electrocardiographic parameters were noted in this study.

Hematology: Treatment related effects on hematologic parameters were noted in low, mid and high dose animals.

Increased white blood cells and reticulocytes were noted in mid and high dose animals. Decreased erythrocytes were noted in mid and high dose animals. Increased absolute lymphocytes and absolute monocytes were noted in low, mid and high dose animals. Increased aPTT was noted in mid and high dose animals

Bone marrow smears from nine monkeys (4 mid dose monkeys, 5 high dose monkeys) were diagnosed with lymphoproliferative disease (equivalent to lymphoma). Three of these monkeys had concurrent leukemia (1 mid dose animal and 2 high dose animals). Increased numbers of stimulated lymphocytes, immunoblasts, plasmacytoid cells and/or atypical lymphocytes, most prominently monocytoid forms were commonly identified in the blood smears of mid and high dose monkeys.

Clinical chemistry: A treatment related increase in serum globulins with a corresponding decrease in serum albumin was noted in animals diagnosed with lymphoproliferative disorder (3 low dose animals, 5 mid dose animals and 10 high dose animals). No other treatment related effects on clinical chemistry parameters were noted in this study.

No treatment related effects on estradiol and testosterone concentrations were noted in this study. The sponsor probably included these measurements to determine if the treatment related effects on hormone levels noted in rats would be apparent in monkeys, as well.

Urinalysis: No treatment related effects on urinary parameters were noted in this study.

Gross pathology: Treatment related macroscopic effects were noted in low, mid and high dose animals. Several animals at doses ≥ 15 mg/kg/day had masses, nodules, thickened, enlarged or discolored organs. Many of these macroscopic effects were due to the diagnosis of lymphoproliferative disorder.

Organ weights: No treatment related changes in organ weights were noted in this study.

Histopathology: Treatment related lymphoid depletion/atrophy and increased macrophages in the thymus, decreased germinal center formation in the spleen and depletion of lymph nodes was present in low, mid and high dose animals. Treatment related morphological changes included a spectrum of lymphoproliferative lesions that were diagnosed based on an adaptation of diagnostic criteria for posttransplant lymphoproliferative disorders (Harris, 1997). The diagnostic criteria for immunosuppressive related lymphoproliferative disorder (IRLD) used in this study are provided in the following table.

Category	Features
Early lesion	Some architectural preservation, immunoblasts along with plasma cells and lymphocytes.
IRLD, polymorphic	Destruction of underlying architecture, shows full range of B cell maturation, may have monomorphic areas.
IRLD, monomorphic	Architectural effacement or invasive growth with confluent sheets of transformed cells. However, monotony does not mean lack of pleomorphism.
IRLD, "other" Hodgkin disease-like	Background of lymphocytes, varying number of granulocytes and presence of Reed Sternberg cells.

The incidence of animals with immunosuppressive related lymphoproliferative disorders (IRLD) is provided in the following table.

Dose (mg/kg)	Lesion Category							
	Polymorphic		Monomorphic		Other (Hodgkin Disease-like)		Total	
	Males	Females	Males	Females	Males	Females	Males	Females
0	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
15	1/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
45	1/4	1/4	1/4	1/4	1/4	0/4	3/4	2/4

120	1/4	2/4	2/4	1/4	0/4	0/4	3/4	3/4
120 ^a	0/2	1/2 ^b	0/2	0/2	0/2	0/2	0/2	1/2 ^b

a – high dose recovery group

b – presented to necropsy on day 6 of recovery

Monomorphic lesions were noted in large intestine, lung, digestive tract, and multi-organ. Polymorphic lesions were noted in spleen, respiratory tract, liver and lung. The primary affected immune cell was the B cell for monomorphic and polymorphic lesions. One instance of Hodgkin disease-like diagnosis was noted in the kidney, with the effected cell being the RS cell. All of the lesions were EBER positive (Epstein Barr virus related).

It is important to note that in human patients undergoing immunosuppressive therapy to prevent transplant organ rejection, post-transplant lymphoproliferative disorder may be fatal even at the early lesion stage. It is possible that the lesion may go into remission (following reduction of immunosuppression with or without chemotherapy) in most stages.

The study report states that these changes were associated with a latent infection by a lymphocryptovirus (LCV). In addition, changes in the epithelium of oral mucosa (tongue or cheek pouch) and esophagus were due to the active, lytic infection by the same virus. No morphological evidence of an LCV infection was noted in control animals or in recovery high dose animals presented to necropsy during week 40 of the study (after 20 weeks of recovery). According to the study report, control and recovery animals were either negative for EBER or had hybridization products in individual cells of spleen and lymph nodes. One of the recovery males had a small cluster of hybridization in a retropharyngeal lymph node that morphologically exhibited mild reactive hyperplasia, but had no evidence of IRLD. Other opportunistic infections noted occasionally in animals included candidiasis of the oral mucosa (tongue, cheek pouch or soft palate) or esophagus and protozoiasis (amoebiasis and spironucleosis) of the large intestine. A single high dose animal had candidiasis after the recovery phase at the week 40 necropsy.

Toxicokinetics: The summary of plasma pharmacokinetic parameters (mean \pm SD) is provided in the following table (n = 4 unless otherwise noted in table).

Dose (mg/kg/day)	Sampling time	AUC ₀₋₂₄ (ng·hr/ml)		C _{max} (ng/ml)		T _{max} (hr)	
		Males	Females	Males	Females	Males	Females
15	Day 1-2	1250 \pm 268	1080 \pm 177	120 \pm 51	122 \pm 41	3 \pm 1.2	4 \pm 0
45		4960 \pm 647	3340 \pm 654	466 \pm 205	283 \pm 88	4 \pm 2.8	3.5 \pm 1
120		7870 \pm 3390	5890 \pm 2330	824 \pm 414	728 \pm 463	3.5 \pm 1	5 \pm 2
15	Week 4	1220 \pm 241	1040 \pm 439	132 \pm 20	136 \pm 59	2 \pm 0	1.8 \pm 0.5
45		4120 \pm 1150	4660 \pm 1170	258 \pm 148	509 \pm 174	2.5 \pm 1.0	4 \pm 0
120		11700 \pm 6160	9170 \pm 2780	1380 \pm 407	1040 \pm 310	3.0 \pm 1.2	3 \pm 1.2
15	Week 38/39	1480 \pm 642	1090 \pm 352	146 \pm 39	104 \pm 35	1.8 \pm 0.5	1.5 \pm 0.6
45		3910 ^a	2680 \pm 467 ^b	420 ^a	296 \pm 47 ^b	2 ^a	1.3 \pm 0.6 ^b
120	Week 19	5220 \pm 806	5060 \pm 1140	589 \pm 148	681 \pm 160	1.8 \pm 1.5	2 \pm 0

a – mean only (n = 2)

b – n = 3

An increase in systemic exposure (AUC and C_{max}) was noted with increased dose, which appeared to plateau at the highest dose indicating a potential saturation of absorption at the highest dose. No apparent sex-related difference in systemic exposure was noted in this study. No apparent increase in systemic exposure with repeat dose was noted in this study.

2.6.6.4 Genetic toxicology

No nonclinical genetic toxicology studies were included in this submission.

2.6.6.5 Carcinogenicity

No nonclinical carcinogenicity studies were included in this submission.

2.6.6.6 Reproductive and developmental toxicology

No nonclinical reproductive and developmental toxicology studies were included in this submission.

2.6.6.7 Local tolerance

No nonclinical local tolerance studies were included in this submission.

2.6.6.8 Special toxicology studies

No nonclinical special toxicology studies were included in this submission.

2.6.6.9 Discussion and Conclusions

A NOAEL for lymphoma (Immunosuppression related lymphoproliferative disorder; IRLD) was not established in the 9 month oral pimecrolimus monkey toxicology study.

The formation of lymphoma is not the only potential cancer concern associated with long term immunosuppressant therapy. It has been demonstrated that kidney and liver transplant patients that receive immunosuppressant therapy (i.e., cyclosporine or tacrolimus) have a higher incidence of skin cancer. Although squamous cell carcinoma is the most common cutaneous malignancy, basal cell carcinoma, melanoma, Kaposi's sarcoma and some uncommon skin malignancies have been reported to occur in this population.

It is recommended that the results of the 9 month oral monkey study be incorporated into the Elidel cream label. The division has concern that it may be possible for lymphoma to be formed after long term topical use of Elidel cream for the treatment of atopic dermatitis.

The low dose in the 9 month oral monkey study is ~30X the maximum proposed topical human dose for Elidel cream for the treatment of atopic dermatitis based on AUC comparisons

(1132 ng·hr/ml ÷ 38 ng/ml·hr = 29.7). The relatively large multiple of human exposure (30X) for lymphoma formation in monkeys compared to the maximum proposed topical human Elidel cream dose might suggest some comfort margin. However, the biologic plausibility of lymphoma formation in local lymph nodes after long term treatment with Elidel cream can not be ruled out at this time. It is acknowledged that demonstrating this effect could be technically challenging. The results of the 9 month oral monkey study demonstrated that long term treatment with oral pimecrolimus caused formation of lymphoma via the same mechanism that has been described in the literature for transplant patients treated with a chronic immunosuppressive regimen (lymphoma formation due to Epstein Barr virus).

2.6.6.10 Tables and Figures – N/A

2.6.7 TOXICOLOGY TABULATED SUMMARY – N/A

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary:

A NOAEL for lymphoma formation (i.e., immunosuppression related lymphoproliferative disorder) was not established in the 9 month oral monkey toxicology study conducted with pimecrolimus.

It is recommended that the results of the 9 month oral monkey study be incorporated into the Elidel cream label.

APPENDIX/ATTACHMENTS

References:

Harris NL, Ferry JA, Swerdlow SH (1997) Posttransplant lymphoproliferative disorders: Summary of Society for Hematopathology Workshop. Semin. Diagn. Pathol. 14: 8-14.