

CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number NDA 50-777

PHARMACOLOGY REVIEW(S)

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

KEY WORDS: Immunosuppressant, Atopic Dermatitis, Dermal Carcinogenicity Study

Reviewer Name: Barbara Hill

Division Name: Dermatologic and Dental Drug Products

HFD#: HFD-540

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NDA number: 50-777

Serial number/date/type of submission: BZ / 6-3-00/ Response to Requested Information

Information to sponsor: Yes No

Sponsor: Fujisawa Healthcare, Inc.
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Deerfield, IL 60015-2548
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Manufacturer for drug substance: Fujisawa Healthcare, Inc.
3125 Staley Road
Grand Island, NY 14072

Drug:

Code Name: FR900506 ointment

Generic Name: FK-506 ointment

Trade Name: Protopic (Tacrolimus) ointment

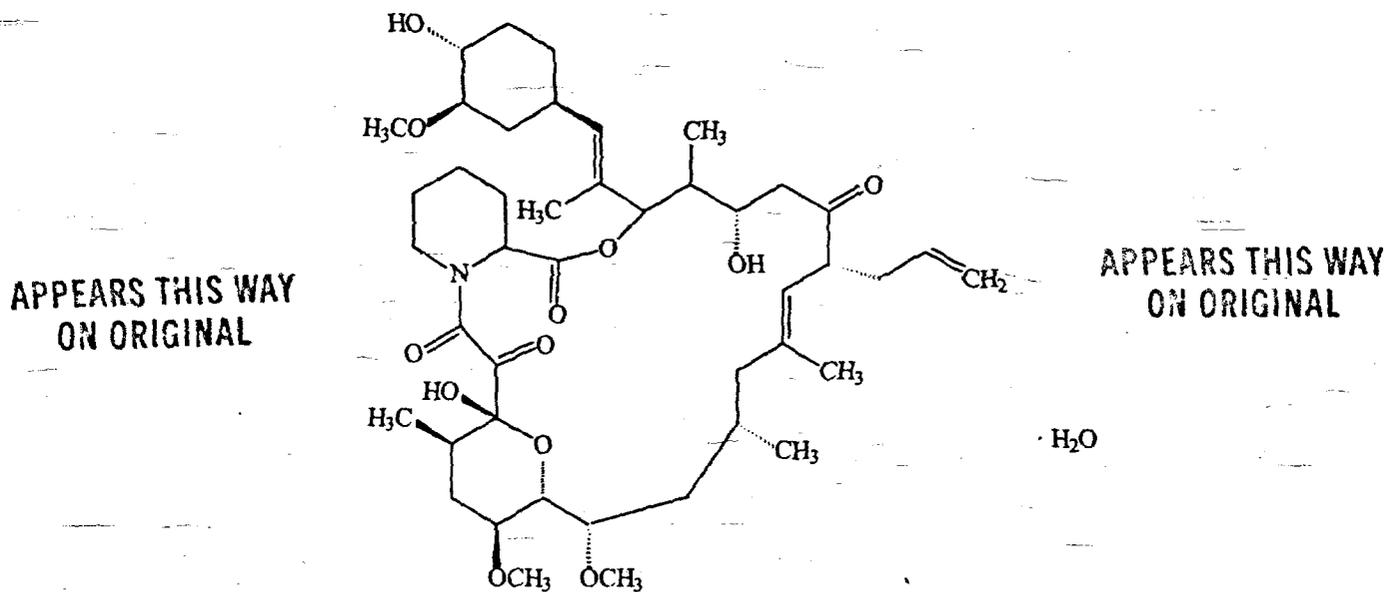
Chemical Name: [3S-[3R*[E(1S*,3S*,4S*)], 4S*, 5R*, 8S*, 9E, 12R*, 14R*, 15S*, 16R*, 18S*, 19S*, 26aR*]]-5, 6, 8, 11, 12, 13, 14, 15, 16, 17, 18, 19, 24, 25, 26, 26a-hexadecahydro-5,19-dihydroxy-3-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethenyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(2-propenyl)-15,19-epoxy-3H-pyrido[2,1-c][1,4] oxazacyclotricosine-1,7,20,21(4H,23H)-tetrone, monohydrate

CAS Registry Number: 104987-11-3

Molecular Formula/ Molecular Weight: C₄₄H₆₉NO₁₂ • H₂O / 822.05

UV Absorption: λ_{max} (1:1,000 dil in methanol): — nm

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Structure:**Relevant INDs/NDA/DMFs:**

- 1) NDA 50-708 (Prograf capsules for prophylaxis of organ {liver} rejection; HFD-590)
- 2) NDA 50-709 (Prograf injection for prophylaxis of organ {liver} rejection; HFD-590)
- 3) IND _____
- 4) IND _____
- 5) IND _____ (Tacrolimus ointment for Atopic Dermatitis; HFD-540)

Drug Class: Macrolide immunosuppressant

Indication: Moderate to severe Atopic Dermatitis

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Dose:

The proposed dose in adults is 0.1% and in pediatric patients is 0.03% _____

Route of administration: Topical dermal

Disclaimer: Note some material may be taken directly from sponsor's submission.

Introduction and drug history:

A topical formulation of tacrolimus, Protopic® (tacrolimus) ointment, has been developed by Fujisawa for treatment of atopic dermatitis. A dermal carcinogenicity study in mice was

conducted with the tacrolimus ointment. A statistically significant increase in lymphoma was noted in this study. Both the trend and pairwise statistical comparison performed by the agency's biostatistical reviewer demonstrated that the incidence of pleomorphic lymphoma was statistically significant in high dose male (25/50) and female animals (27/50) and that the incidence of undifferentiated lymphoma was statistically significant in high dose female animals only (13/50). The results from this 2 year dermal carcinogenicity study were presented to the Executive CAC on 3/14/00. The Executive CAC recommendations and conclusions are presented below.

Executive CAC Recommendations and Conclusions:

1. The committee determined that the mouse dermal carcinogenicity study was adequate and that there was a strong signal for lymphoma.
2. The committee requested historical background incidence rates for hepatocarcinoma, stromal cell sarcoma in the cervix and leiomyoma in the uterus for the strain of mouse used in the dermal carcinogenicity study. A request will be sent to the sponsor for these historical background incidence rates.
3. The committee requested clarification whether it was known if the lymphomas noted in this study were of a B-cell or T-cell origin. A request will be sent to the sponsor to determine if this information is known.
4. The committee strongly recommended that if tacrolimus ointment is approved, then the division should consider strong label warnings for the potential lymphoma risk and photocarcinogenic risk associated with tacrolimus ointment use. Also, the committee recommended that wording be added in the label to indicate that even though no skin cancer was noted in the mouse dermal carcinogenicity study, that there may still be a risk in humans due to the presence of human papilloma virus in humans that was not present in mice. It was noted that the exposure at the NOEL for lymphoma were significantly closer to those produced in human at the recommended dose and that this should be considered in the risk benefit as well as the presence of tumors (still of questionable significance) in the lowest dose tested.

A meeting was conducted with the review team for this NDA to discuss the results of the 2 year mouse dermal carcinogenicity study on 3-29-00. The result of this meeting was a letter sent to the sponsor (via FAX on 3-30-00) requesting additional clinical data, clinical pharmacology and nonclinical data to address the potential lymphoma risk possibly associated with the use of tacrolimus ointment.

The sponsor was informed by tele-conference (3-30-00) that they would be receiving this FAX. In addition, the sponsor was informed that the concern about lymphoma formation after topical use of tacrolimus ointment in atopic dermatitis patients would be discussed in an upcoming Advisory Committee meeting.

The current submission is a response to the Clinical, Biopharmaceutics and Pharmacology/Toxicology questions sent to the sponsor. The adequacy of the sponsor's reply to the Pharmacology/Toxicology questions will be discussed in the next section of this review. In

addition, discussion of the sponsor's reply to the clinical and biopharmaceutics issues raised will also be discussed in relation to Pharmacology/Toxicology concerns.

Review of Sponsor's Reply to Pharmacology/Toxicology Questions:

Each question will be reproduced below followed by the sponsor's reply to the question. This will be followed by the reviewer's comments as to the adequacy of the sponsor's response.

Note: The sponsor states that a white paper which presents the available human data for the tacrolimus ointment trial as well as relevant data from oral and topical animal studies with tacrolimus is part of this submission. The sponsor stated that this information would be useful in the review of their responses to the following questions. This information was considered in my review of the sponsor's reply to the following questions.

Question 1:

Please provide historical background incidence rates from the contract laboratory that conducted the 2-year mouse dermal carcinogenicity study for tacrolimus ointment for the following tumor types:

Liver-Carcinoma

Cervix-Stromal cell Sarcoma

Uterus-Leiomyoma

Sponsor's Response to Question 1:

The historical tumor incidence for liver carcinomas, cervical stromal cell sarcomas, and uterine leiomyomas for the contract laboratory (_____) where the 2-year topical B6C3F1 mouse carcinogenicity study was conducted with tacrolimus ointment is presented in Table 3.1. For comparison, Table 3.1 also contains comparable historical data from the National Toxicology Program as well as the incidence for these tumors observed in the 2-year topical study. In addition, the incidence for liver carcinomas, cervical stromal cell sarcomas, and uterine leiomyomas in a 78-week oral CD-1 mouse carcinogenicity study (Table 3.2) and in a 2-year oral CD rat carcinogenicity study (Table 3.3) conducted with tacrolimus are provided for comparison. The reports of these studies were part of NDA 50-708/50-709 (Prograf[®], capsules and injection); a comprehensive summary of the rodent carcinogenicity data has also been submitted to this NDA (NDA 50-777) and is available.

The data for the 2-year B6C3F1 mouse topical study (see Table 3.1) indicates that the incidence of all three tumor types for Group 3 (0.03% ointment, equivalent to 1.6 mg/kg/day) are above the laboratory historical levels as well as the concurrent study controls, but none of the tumor types are statistically elevated by the Peto analysis compared to control groups in the study. Also, the lower incidence of all three tumors in Group 4 (the highest surviving dose level; 0.1% ointment equivalent to 5.3 mg/kg/day) indicates that a dose-response is absent. Although survival (Table 3.4) in Group 4 of the topical study was lower compared to Groups 1-3, it is considered within the acceptable range for evaluation of tumor development since 40% of both

sexes survived to month 24. The systemic exposure of Group 3 in the topical study should be viewed as equivalent to the systemic exposure in Group 5 (3.0 mg/kg/day) of the oral mouse study as evidenced by the AUC for both studies (Table 3.5). Although the duration of the two mouse studies and the intervals at which the TK samples were collected were not identical, there is no evidence (from these and a number of other rodent toxicity and ADME type studies) of accumulation of tacrolimus in the blood with repeated dosing. Therefore, it is possible to compare the toxicokinetic data and tumor incidence of the various studies. It should be noted that in the presence of similar blood concentrations of tacrolimus (Table 3.5), the incidence of the three tumor types in the oral CD-1 mouse study are not elevated compared to the respective study controls. Also, the incidence of these three tumor types among the tacrolimus treated groups were not different from controls in the 2-year oral rat study. Consequently, the incidence of the three tumor types is not supported by similar carcinogenicity studies in the CD-1 mouse or the CD rat.

In summary, even though the incidence of liver carcinomas, cervical stromal cell sarcomas, and uterine leiomyomas in the 0.03% Group of the 2-year B6C3F1 topical mouse study are numerically higher than the controls, these findings are:

- not statistically significantly different from the controls based on Peto analysis
- not dose related
- not observed in an oral mouse carcinogenicity study with similar systemic exposure to tacrolimus
- not observed in an oral rat carcinogenicity study up to and including the MTD with tacrolimus indicating a species and strain specificity to mice (i.e., B6C3F1)

Table 3.1: Historical Control Incidence for Selected Tumors Relative to the B6C3F1 Mouse in 2-Year Topical Carcinogenicity Study

	Historical Controls		2-Year Topical Tacrolimus Ointment CA			
			Group			
	Untreated	NTP** Untreated	1 Untreated	2 Vehicle	3 0.03%	4 0.1%
Dose Level (mg/kg)				0	1.6	5.3
Liver						
carcinoma (m)	20.0% (30/150) (range: 16.0 – 24.0%)	17.9% (241/1350) (range: 6 – 29%)	10% (5/50)	12% (6/50)	28% (14/50)	8% (4/50)
carcinoma (f)	12.1% (18/149) (range: 10.2 – 14.0%)	8.4% (113/1350) (range: 0 – 20%)	6% (3/50)	8% (4/50)	2% (1/50)	4% (2/50)
Uterus						
leiomyoma	0.7% (1/149) (range: 0.0 – 2.0%)	0.5% (7/1353) (range: 0 – 4%)	0% (0/50)	0% (0/50)	6% (3/50)	2% (1/50)
Cervix						
stromal sarcoma	0.0% (0/149)	0.4% (5/1353) (range: 0 – 2%)	0% (0/50)	0% (0/50)	10% (5/50)	0% (0/50)

* Personal communication, Dr. Joseph Haseman, National Toxicology Program, April 4, 2000.

** Haseman, J.K., Hailey, J.R., and Moris, R.W. Spontaneous neoplasm incidences in Fischer 344 rats and B6C3F1 mice in two-year carcinogenicity studies: A National Toxicology Program Update, Toxicologic Pathol 26: 428-441, 1998.

Table 3.2: Tumor Incidence for CD-1 Mouse in 78-Week Oral (Dosed Feed) Carcinogenicity Study

Group	Group					Historical Control
	1	2	3	4	5	
Dose Level (mg/kg)	-----	0	0.3	1.0	3.0	Untreated
Liver						
carcinoma (m)	0%	7.1%	8.9%	3.6%	1.8%	4.9% (range: 1.3 - 11.5%)
carcinoma (f)	0%	0%	0%	0%	0%	-----
Uterus						
leiomyoma	0%	2.6%	2.1%	2.6%	2.4%	1.7% (range: 0 - 3.9%)
Cervix						
stromal sarcoma	0%	5.3%	0%	0%	0%	0.5% (range: 0 - 6.0%)

Table 3.3: Tumor Incidence for CD Rat 2-Year Oral (Dosed Feed) Carcinogenicity Study

Group	Group								Historical Control
	1	2	3	4	5	6	7	8	
Dose Level (mg/kg)	-----	0	0	0.2	0.5	1.25	2.5	5.0	Untreated
Liver									
carcinoma (m)	3.6%	0%	2.0%	0%	0%	0%	0%	0%	2.6% (range: 0-9.1%)
carcinoma (f)	0%	0%	0%	1.8%	0%	3.6%	0%	0%	0.4% (range: 9-4.0%)
Uterus									
leiomyoma	0%	0%	0%	0%	0%	0%	0%	0%	--
Cervix									
stromal sarcoma	0%	0%	0%	0%	0%	0%	0%	0%	--

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Table 3.4: Survival (%) Comparison of B6C3F1 Mice, CD-1 Mice and CD Rats in Carcinogenicity Studies with Topical or Oral FK506

2-Year Topical B6C3F1 Mouse Study								
Group	1	2	3	4	5	6	7	8
Ointment (%)	--	Vehicle	0.03	0.1	0.3	1	3	
Dose Level (mg/kg)	--	0	1.6	5.3	15.8	52.6	157.9	
Male	84	82	78	42	14	0	0	
Female	82	88	78	40	16	0	0	
78-Week Oral CD-1 Mouse Study								
Group	1	2	3	4	5			
Ointment (%)	--	--	--	--	--			
Dose Level (mg/kg)	Basal Diet	0	0.03	1.0	3.0			
Male	66	82	77	73	55			
Female	77	68	84	70	75			
2-Year Oral CD Rat Study								
Group	1	2	3	4	5	6	7	8
Ointment (%)	--	--	--	--	--	--	--	--
Dose Level (mg/kg)	Basal Diet	0	0	0.2	0.5	1.25	2.5	5.0
Male	64	73	56	72	71	78	54	0
Female	53	69	64	58	62	60	70	64

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Table 3.5: Blood Concentration (AUC_{0-24hr}: ng·hr/ml) of FK506

2-Year Topical B6C3F1 Mouse Study							
Group	1	2	3	4	5	6	7
Ointment (%)	--	Vehicle	0.03	0.1	0.3	1	3
Dose Level (mg/kg)	--	0	1.6	5.3	15.8	52.6	157.9
Male/Female Combined ^a	--	--	202	536	894	2290	11992
Male/Female Combined ^b	--	--	114	466	--	--	--
13-Week Oral CD-1 Mouse Study							
Group	1	2	3	4	5		
Ointment (%)	--	--	--	--	--		
Dose Level (mg/kg)	Basal	Placebo	0.03	1.0	3.0		
Male/Female Combined ^c	--	--	12	33	182		

- a: TK sample collection point – 6 months
b: TK sample collection point – 18 months
c: TK sample collection point – 13 weeks

Reviewer's Comments for Sponsor's Response to Question 1:

The incidence of liver carcinoma (28%) in the low dose group (0.03%) of the mouse dermal carcinogenicity study is within the historical range for male mice based on the NTP database (range: 0 – 29%) but not for the contract lab database (range: 0 – 24%). Liver carcinoma in control male mice is a relatively common event that occurs during a 2 year carcinogenicity study. It is my opinion that even though the low dose group incidence rate was relatively high, it is not biologically significant since this is a relatively common tumor in male mice and it did not demonstrate a dose response relationship in this study (high dose {0.1%} incidence = 8%).

The incidence of uterine leiomyoma (6%) in the low dose group (0.03%) of the mouse dermal carcinogenicity study was not within the historical range for mice based on either the NTP database (range: 0 – 4%) or the contract lab database (range: 0 – 2%). It is my opinion that even though the low dose group incidence rate was higher than both historical range values, it is still not biologically relevant for tacrolimus. The rationale for this decision is that the 6% incidence rate is only 2% above the NTP historical range and no dose response was demonstrated in this study (high dose incidence {0.1%} = 2%).

The incidence of cervix stromal sarcoma (10%) in the low dose group (0.03%) of the mouse dermal carcinogenicity was not within the historical range for mice based on either the NTP database (range: 0 - 2%) or for the contract lab database (range: 0%). It is my opinion that even though the low dose group incidence rate was higher than both historical range values, it is still not biologically relevant for tacrolimus. The main rationale for this is that no dose response was demonstrated in this study (high dose incidence {0.1%} = 0%). It is interesting to note that the incidence of cervix stromal sarcoma was 5.3% for the vehicle control group in the oral tacrolimus CD-1 mouse study. Even though this incidence rate was seen in a different strain of mouse, it may indicate that the incidence of cervix stromal sarcoma may be more variable than indicated by the historical range data.

The most striking and biologically relevant finding in the mouse dermal carcinogenicity study conducted with tacrolimus ointment was an increase in lymphoma. A statistically significant elevation in the incidence of pleomorphic lymphoma in high dose male (25/50) and female animals (27/50) and in the incidence of undifferentiated lymphoma in high dose female animals (13/50) was noted in the mouse dermal carcinogenicity study. This is the information that was recommended for inclusion in the Protopic label in my original NDA review. It is still my opinion that this is the most significant tumor finding for tacrolimus ointment. It is not recommended that the incidence rates for liver carcinoma, uterine leiomyoma or cervix stromal sarcoma be included in the label because their biological relevance is questionable.

It is important to note that the mouse oral (feed) carcinogenicity study was inadequate for assessing potential human risk. The duration of the study was not the accepted agency standard (78 weeks vs 104 weeks). The decreased duration of this study is significant because the majority of the lymphomas noted in the 2 year mouse dermal carcinogenicity study were noted in the last quarter of the study (weeks 78-104). Therefore, the 78 week mouse oral (feed) carcinogenicity study was ended just prior to the point where a significant increase in lymphomas may have been noted in this study. Another important point is that the total exposure to tacrolimus was much lower in the 78 week mouse oral (feed) carcinogenicity study versus the 2-year mouse dermal carcinogenicity study. This could have a significant impact on formation of lymphoma in the mouse model. The 78 week mouse oral (feed) carcinogenicity study was conducted in a different strain of mouse (CD-1 mouse) than the 2 year mouse dermal carcinogenicity study (B6C3F1 mouse). This could be significant in reference to the background incidence of lymphoma for a particular strain of mouse. For example, after 78 weeks it may not be possible to differentiate between the lymphoma background rate and treatment related lymphoma for CD-1 mice. However, if the study had been extended to 104 weeks, it may have been possible to detect a treatment related increase in lymphoma over the background rate. Taking into consideration all of these factors, the mouse oral (feed) carcinogenicity study was inadequate for a negative study due to an inadequate duration of the study (78 weeks instead of 104 weeks) with a corresponding decrease in total exposure to tacrolimus.

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Question 2:

The Sponsor should clarify whether the lymphomas noted in the 2-year mouse dermal carcinogenicity study conducted for tacrolimus ointment were of a B-cell or T-cell origin, if know.

Sponsor's Response to Question 2:

An assessment of the B-cell or T-cell origin of the lymphomas of each animal in the 2-year dermal mouse carcinogenicity study has been conducted by a consulting pathologist (____ D.V.M, Ph.D). His assessment indicates that the great majority of the lymphomas found were of B-cell origin, specifically follicular center cell lymphomas. The proportion of B-cell lymphomas across the evaluable dose groups is shown in Table 3.6.

Table 3.6: Proportion (%) of Lymphomas Identified as B-cell Lymphomas in B6C3F1 Mice Topically Treated with Tacrolimus Ointment for 2-Years

	Group			
	1	2	3	4
Male	83.3 (5/6)	75.0 (3/4)	50.0 (4/8)	90.6 (29/32)
Female	63.2 (12/19)	88.9 (8/9)	77.3 (17/22)	86.7 (39/45)

() = B-cell lymphomas / total number of lymphomas

Follicular center cell lymphoma is one of the most common spontaneous lymphomas in mice. It is more common in females than males, and in some strains the incidence may reach 50% [Frith, CH and Wiley, JM (1981)]. It should be pointed out the lymphomas found in mice are not necessarily indicative of an increased lymphoma risk in humans, as the problem in immunosuppressed transplant patients has been EBV - related PTLD in the vast majority of cases. EBV is a human, not a mouse virus. These animal studies are therefore not informative (either positively or negatively) about the risk of EBV - related lymphoproliferation resulting from topical tacrolimus (personal communication, _____ Mary 26, 2000).

Reviewer's Comments for Sponsor's Response to Question 2:

A related question posed by clinical was for the sponsor to clarify whether the lymphomas noted in recipients of Prograf were of a B-cell or T-cell origin. The sponsor replied that posttransplant lymphoproliferative disorders (PTLD) are generally B-cell in origin. The distribution of lymphomas with respect to B-cell or T-cell origin based on the 215 cases of PTLD identified in the Fujisawa global safety data base for Prograf therapy is provided in the following table.

Lymphoma Type	Number of reported cases
B-cell	87
T-cell	6
B/T cell	16
Type unknown	35
Not confirmed	71

Based on Fujisawa global safety data base

Based on this data, the sponsor claims that the experience with Prograf is consistent with the literature on PTLD. It would also appear that the type of lymphoma noted in the 2-year mouse dermal carcinogenicity study (mainly B-cell lymphoma) was consistent with the type of lymphoma noted in Prograf patients (mainly B-cell lymphoma). The sponsor's argument that the lymphomas found in mice are not necessarily indicative of an increased lymphoma risk in humans since the problem in immunosuppressed transplant patients has been EBV - related PTLD in the vast majority of cases is not an accurate representation of the situation. It is true that EBV is a human virus and not a mouse virus. It may also be true that the animal carcinogenicity study will not address the concern of EBV related lymphoma specifically for humans. However, the strong signal for lymphoma noted in the mouse dermal carcinogenicity study might be cause for significant concern for lymphoma in humans.

The argument that the mechanism for the development of lymphoma may be different between humans and animals and therefore a positive signal in animals is not cause for concern is flawed for the following reason. It has not been assured that the absolute mechanism for formation of lymphoma in humans after immunosuppressive therapy has been totally defined yet. An example of this relates to a recent published article in nature titled "Cyclosporine induces cancer progression by a cell-autonomous mechanism"¹. The results from the studies conducted in this article suggest that cyclosporine may have two potential mechanisms for tumor progression. The most common mechanism proposed is that cyclosporine causes a decrease in T-lymphocyte function which leads to systemic immunosuppression and a corresponding increase in the lymphoma formation due to a host decrease in immunosurveillance potential. A second hypothesis proposed from the results of the studies in this article is that cyclosporine treatment causes an increase in TGF- β , which may lead to malignant transdifferentiation of a non-invasive tumor to an invasive carcinoma. An increase in TGF- β was noted in cyclosporine treated adenocarcinoma cells. The increase in TGF- β expression corresponded with striking morphological alterations that were indicative of potential increased invasiveness of this tumor cell line. In addition, *in vivo* data demonstrated that cyclosporine promoted tumor growth in SCID-beige mice (which are deficient in T cells, B cells and natural killer cells) and anti-TGF- β antibodies prevented the cyclosporine induced increase in metastases. These findings suggest that immunosuppressants like cyclosporine (or possibly tacrolimus) can promote cancer progression by a direct cellular effect that is independent of its effect on the host's immune cells and that cyclosporine induced TGF- β production may be involved in the direct cellular effect.

¹ Hojo M, Morimoto T, Maluccio M, Asano T, Morimoto K, Lagman M, Shimbo T and Suthanthiran M. (1999) Cyclosporine induces cancer progression by a cell-autonomous mechanism. Nature 397: 530-534.

Therefore, it is not possible to argue that the results from the mouse carcinogenicity study are not important for evaluating the human risk of development of lymphoma after long term tacrolimus therapy. The lymphoma formation noted in the mouse dermal carcinogenicity study is a strong signal that indicates that there might be significant risk for development of lymphoma in humans. From a Pharm/Tox basis, the level of risk can be estimated by examining the difference in fold exposure to tacrolimus in the mouse dermal carcinogenicity study and in humans after treatment with the maximum anticipated human dose for tacrolimus ointment. This calculation will be discussed in more detail in the next section of this review.

Question 3:

It is recommended that the Sponsor conduct a nonclinical study in minipigs, or other suitable species, to determine the concentration of tacrolimus in the regional lymph nodes that drain from the skin after topical tacrolimus application to abraded or irritated skin. The purpose of this study is to determine if the concentration of tacrolimus in regional lymph nodes that drain from the skin is higher than or the same as the level of tacrolimus in the blood after topical administration. This information is necessary for the determination of human risk for lymphoma after topical administration of tacrolimus ointment. It is recommended that the Sponsor submit the study protocol for this study to the Division for review prior to initiation of the study.

Sponsor's Response to Question 3:

FHI has performed both single and repeated dose topical studies with ¹⁴C-tacrolimus ointment in rats (Protopic NDA 50-777, Sections 5.3.3.2 and 5.3.3.3, Volume 9). These data demonstrate that after a single application of ointment to intact and abraded skin, the concentration of tacrolimus in lymph (mesenteric node) was greater than in blood. After repeated application for up to 14 days, there was no evidence of an increase in tacrolimus concentration in lymph nodes or in blood.

Fujisawa Healthcare Inc. (FHI) has reviewed and discussed the purpose and conduct of this minipig study. Since the regional lymph nodes drain the dermis and are more proximal to the application sites, we can readily assume that their tacrolimus concentrations would be greater than the concomitant systemic blood concentrations early after the initial application. With repeated application, this relationship changes as tacrolimus partitions from lymph into systemic blood.

Given the above rodent data, we do not feel it is necessary to perform the proposed study since an increase in regional lymph node concentration of tacrolimus relative to whole blood is a logical expectation and is supported by the studies cited above. Even if an increase in regional lymph node tacrolimus concentration of the minipig is demonstrated, we do not believe that this information will contribute to the assessment of a human risk for lymphoma after topical administration of tacrolimus.

Concerns regarding localized lymphomagenesis in regional nodes, on the basis of presumed high local tacrolimus levels is a valid theoretical concern. However, it should be

pointed out that immunodeficiency-related EBV associated lymphoproliferations in humans are known to arise in a multicentric fashion. The fact that PTLD tends to arise simultaneously and independently at multiple sites in the body suggests that the level of overall immune function, rather than local factors, is important. In this context, systemic immunosuppression has not been demonstrated after tacrolimus ointment use.

In summary, we expect that the initial tacrolimus concentration will be greater in regional lymph nodes that drain the skin after topical administration as compared to blood. However, as cited above in the rat study, such concentrations decline and do not accumulate. Also, we are not aware of a relationship between the local lymph node drug concentration and the risk of lymphoma. In addition, the topical application of tacrolimus to humans does not result in concentrations of tacrolimus that are associated with systemic immunosuppression. In view of all of the above considerations, we propose that it is unnecessary to conduct the minipig study.

Reviewer's Comments for Sponsor's Response to Question 3:

The sponsor's representation of the results of the pharmacokinetic rat study conducted with tacrolimus ointment is accurate. It should be noted that this study was not undertaken to specifically determine the level of tacrolimus in lymph nodes vs blood but was performed as a general absorption, distribution and elimination study for tacrolimus ointment. However, the results do demonstrate that after topical administration of tacrolimus ointment to rats the levels of tacrolimus were higher in lymph nodes than in blood.

In addition, the sponsor does admit that they believe that the level of tacrolimus is likely to be higher in local lymph nodes than blood after topical administration of tacrolimus ointment to humans. The sponsor also states that they are not aware of a relationship between local lymph node drug concentration and the risk of lymphoma. Simply because the sponsor is not aware of a relationship does not mean that a potential one does not exist. Since the sponsor has demonstrated in the rat that local lymph node exposure is greater than general systemic exposure (blood) after topical application of tacrolimus ointment, it is unclear how much additional useful information can be obtained from the conduct of the minipig study. Even if the sponsor does conduct this study and finds higher lymph node levels than in the blood, it is unclear how to relate this to a potential increase in lymphoma risk based on the increased level at this time. Therefore, it is recommended that the sponsor does not need to conduct the recommended study at this time. If additional information should become available in the future to help us better understand local lymph node levels and potential lymphoma risk, then this issue will need to be revisited at that time.

Evaluation of human lymphoma risk from a Pharm/Tox perspective:

The sponsor states that the incidence of PTLD is linked to intense immunosuppression and patient risk factors such as EBV. It is unclear what the meaning is of intense immunosuppression and what methods were used to assess the level of immunosuppression in human patients. The sponsor provides estimates of fold exposure between atopic dermatitis patients and transplant recipients and transplant recipients who develop PTLD. The use of this

data for risk assessment for lymphoma will be a task for the medical officer. The estimate of risk of lymphoma development from a pharm/tox perspective is based on the fold AUC exposure level comparison between mice with lymphoma and humans under maximum use conditions.

One factor that the sponsor brings up for consideration of ultimate lymphoma risk is that clinical data support that quantifiable blood concentrations are not observed for prolonged periods. Exposure to quantifiable blood levels of tacrolimus in atopic dermatitis patients is transient and generally restricted to periods when lesions are present. Absorption decreases concurrently with lesion healing. The sponsor states that affected body surface area decreases rapidly usually within 1 week of the start of treatment with tacrolimus ointment.

The sponsor states that in general, transplant patients receive doses of Prograf (recommended range: 0.1-0.2 mg/kg/day) so that they maintain a minimum (trough) whole blood concentration of ~10 ng/ml over the course of their lives. In comparison, the majority of atopic dermatitis patients do not experience absorption into the systemic circulation. For those who have a quantifiable concentration, these levels are observed only for a short, isolated period of time, usually early in treatment before lesions heal. The sponsor claims that less than 2% of patients in the world wide development of tacrolimus ointment had isolated levels approaching the targeted trough levels observed in transplant patients.

The sponsor claims that there is no clinical evidence of either systemic immunosuppression nor lymphoproliferative disorders in atopic dermatitis patients based on a large patient base (6,906). The manner in which systemic immunosuppression was assessed is not stated in the submission. It is not totally reassuring that no lymphoproliferative disorders have been seen in atopic dermatitis patients because the length of time of treatment and follow up have not been long enough to assure that the risk is minimal.

It is interesting to note that the sponsor states that while it is known that the risk for PTLD is influenced by the intensity of immunosuppression, it is not possible to predict what constitutes an intense or excessive level of immunosuppression for any individual patient. I would also add that no data is available to support the statement that PTLD development depends on the overall level of immune suppression as opposed to localized factors (e.g., in regional lymph nodes). No data was presented to support this claim and it is unknown what role immunosuppression in the regional lymph nodes may play in formation of PTLD.

The sponsor states that a major risk factor for PTLD is infection with Epstein-Barr virus (EBV). EBV inserts its DNA into the genome of B-cells of infected individuals. In immunocompetent and many immunocompromised individuals, lymphoproliferation does not occur since the proliferation of these EBV-transformed B-cells is controlled by virus-specific cytotoxic T-cells and natural killer cells. However, it is proposed that this immunosurveillance mechanism can be disrupted by intense immunosuppression or cumulative use of multiple immunosuppressants, leading to the uncontrolled proliferation of transformed B-cells. I propose that there are potentially additional mechanisms for PTLD formation that may be associated with immunosuppressive therapy that are independent of EBV infection (i.e., ↑TGF- β production or

possible immunosuppressive effects on regional lymph nodes). EBV infection is definitely one risk factor, but is potentially not the only risk factor that is associated with PTLD.

The sponsor maintains a database for PTLD associated with Prograf (tacrolimus) use. The number of PTLD cases associated with Prograf for the period March 1989 to March 1999 in this database was 215 (no denominator was provided for this incidence rate). Time to onset of PTLD was recorded in 179 of the 215 cases. Time to onset for adult patient (>15 years of age; n=82) was 230 ± 339 days and for pediatric patients (≤ 15 years; n=97) was 261 ± 298 days. Concomitant immunosuppressant use was high in transplant recipients in the database that developed PTLD. The sponsor states that the incidence of PTLD in clinical studies was 45/5364 (0.8%) as of September 30, 1997. An incidence rate of 0.6% (8/1428) for PTLD in clinical studies was noted during the subsequent 1.5 year period (September 1997 to March 1999).

Oral (dietary) carcinogenicity studies were conducted in rats and mice in support of the Prograf application. No relationship between tumor incidence and tacrolimus dosage was observed in these studies. AUC data was not obtained in either carcinogenicity study. The sponsor conducted 13 week oral (dietary) studies in rats and mice with toxicokinetic analysis as a phase 4 commitment. It was determined that the AUCs determined in these dose range finding studies with dietary administration should be representative of the exposure in the carcinogenicity studies. The same doses used in the carcinogenicity studies were used in the dose range studies. An important point to note is that the mean AUC was less than 250 ng-hr/ml at all time points. In addition, the exposure in mice was ~5-10X the level noted in rats. The AUC value for the 3 mg/kg/day tacrolimus dose (highest dose) from the dietary exposure 13 week mouse study after 14 weeks was 182.4 ng-hr/ml. The AUC value for the 5 mg/kg/day (highest dose) tacrolimus dose from the dietary exposure 13 week rat study after 14 weeks was 53.8 ng-hr/ml.

A mouse dermal carcinogenicity study was conducted to support the Protopic application. The MTD was identified as the 0.1% tacrolimus ointment dose (5.3 mg/kg/day) based on mortality. A statistically significant elevation in the incidence of pleomorphic lymphoma in high dose male (25/50) and female animals (27/50) and in the incidence of undifferentiated lymphoma in high dose female animals (13/50) was noted in the mouse dermal carcinogenicity study. The sponsor states that the lymphoma result is likely related to high systemic exposure resulting from a combination of high cutaneous absorption and ingestion. The animals did not wear "Elizabethan" collars during the study and ingestion of tacrolimus ointment may have occurred during grooming. The sponsor states that they are currently conducting a three group study to further evaluate systemic exposure following topical application in mice wearing "Elizabethan" collars, not wearing "Elizabethan" collars or receiving the ointment orally via gavage. It is important to note that higher systemic exposure was obtained in the mouse dermal carcinogenicity study (average AUC for 0.1% dose = 534 ng-hr/ml) than in either oral rat or mouse carcinogenicity study. This may be the reason that a significant lymphoma incidence was noted in the dermal carcinogenicity study and not in either oral carcinogenicity study.

The sponsor provided data from a European clinical pharmacokinetic study in this submission. This data was supplied due to a direct request from the clinical biopharmacologist. Only the human AUC data from the 0.1% tacrolimus ointment treated patients will be discussed here in relation to the dermal carcinogenicity study AUC data. The rationale for this is that this is the highest concentration to be used clinically and provided for the highest AUC value under maximum use conditions.

The highest mean AUC_{0-12 hr} value observed in the adult study was 10.2 ng-hr/ml on day 4 in the group with 36-60% of body surface area treatment (n=9). This would equal an AUC_{0-24 hr} value of 20.4 ng-hr/ml. The no effect dose AUC in the dermal carcinogenicity study is ~9 fold greater than the maximum human AUC obtained in this study (189 ng-hr/ml ÷ 20.4 ng-hr/ml). The AUC for the dose that lymphomas were noted in the dermal carcinogenicity study is ~26 fold greater than the maximum human AUC obtained in this study (534 ng-hr/ml ÷ 20.4 ng-hr/ml). It is my opinion that human patients would not have a high risk of getting lymphomas under conditions of clinical use for the 0.03% and 0.1% tacrolimus ointment, from a pharmacological/toxicological perspective. This assessment has not changed from the original NDA review.

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/S/

Barbara Hill, Ph.D.
Reviewing Pharmacologist

cc:

NDA: 50-777 (BZ)

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REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

KEY WORDS: Immunosuppressant, Atopic Dermatitis

Reviewer Name: Barbara Hill

Division Name: Dermatologic and Dental Drug Products

HFD#: HFD-540

Review Completion Date: 6-22-00

JUN 27 2000

NDA number: 50-777

Serial number/date/type of submission: 000 / 9-14-99 / Original NDA Submission

Information to sponsor: Yes (X) No ()

Sponsor: Fujisawa Healthcare, Inc.
Parkway North Center, Three Parkway North
Deerfield, IL 60015-2548
(847) 317-8800

Manufacturer for drug substance: Fujisawa Healthcare, Inc.
3125 Staley Road
Grand Island, NY 14072

Drug:

Code Name: FR900506 ointment

Generic Name: FK-506 ointment

Trade Name: Protopic (Tacrolimus) ointment

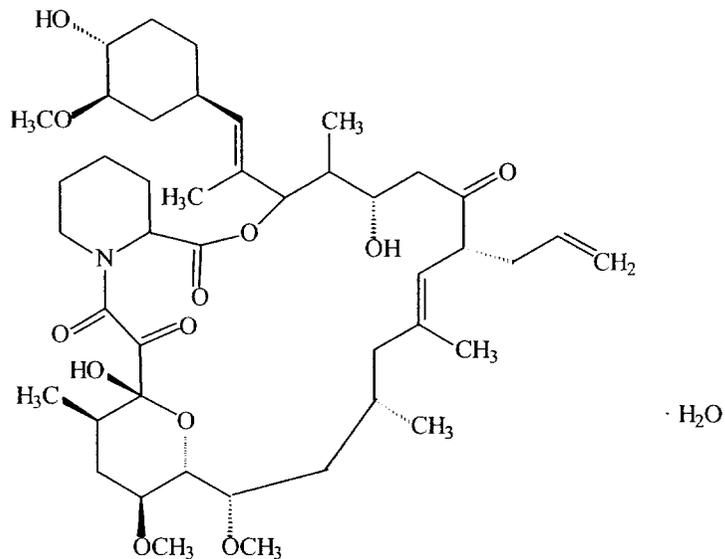
Chemical Name: [3S-[3R*[E(1S*,3S*,4S*)], 4S*, 5R*, 8S*, 9E, 12R*, 14R*, 15S*, 16R*, 18S*, 19S*, 26aR*]]-5, 6, 8, 11, 12, 13, 14, 15, 16, 17, 18, 19, 24, 25, 26, 26a-hexadecahydro-5,19-dihydroxy-3-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethenyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(2-propenyl)-15,19-epoxy-3H-pyrido[2,1-c][1,4] oxaazacyclotricosine-1,7,20,21(4H,23H)-tetrone, monohydrate

CAS Registry Number: 104987-11-3

Molecular Formula/ Molecular Weight: C₄₄H₆₉NO₁₂ • H₂O / 822.05

UV Absorption: λ_{max} (1:1,000 dil in methanol): — nm (active only)

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Structure:**Relevant INDs/NDAs/DMFs:**

- 1) NDA 50-708 (Prograf capsules for prophylaxis of organ {liver} rejection; HFD-590)
- 2) NDA 50-709 (Prograf injection for prophylaxis of organ {liver} rejection; HFD-590)
- 3) IND ~~_____~~
- 4) IND ~~_____~~
- 5) IND ~~_____~~ (Tacrolimus ointment for Atopic Dermatitis; HFD-540)

Drug Class: Macrolide immunosuppressant

Indication: Moderate to severe Atopic Dermatitis

Clinical formulation:

The composition of the test article and vehicle used in Phase 3 clinical studies and nonclinical studies is provided in the following table (the composition of the 0.03% and 0.1% ointment is the same as the to be marketed formulation):

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Substance	Vehicle	0.03%	0.1%	0.3%	1%	3%
FR900506 (Tacrolimus)	┌					
Propylene carbonate						
White wax						
Mineral oil						
Paraffin						
White petrolatum						

Dose:

The proposed dose in adults is 0.1% tacrolimus ointment and in pediatric patients is 0.03% ~~—~~ tacrolimus ointment applied topically twice daily as a thin layer to affected areas of skin. It is estimated that up to 80% of the body could be treated in a severe case of atopic dermatitis. Approximately 30 g of tacrolimus ointment would be applied per treatment to cover 80% of the body. Therefore, the maximum daily dose of the 0.1% tacrolimus ointment would be 1.2 mg/kg/day for a 50 kg person ($30,000 \text{ mg} \times .001 \times 2/\text{day} \div 50 \text{ kg} = 1.2 \text{ mg/kg/day}$).

Route of administration: Topical dermal

Disclaimer: Note some material may be taken directly from sponsor's submission.

Introduction and drug history:

Tacrolimus (also known as FK506) is a 23 member macrolide immunosuppressant produced by *Streptomyces tsukubaensis*, a soil bacterium found in Mount Tsukba, Japan. Tacrolimus inhibits the early activation of T-lymphocytes. Tacrolimus was originally approved in the United States in April 1994 in Prograf[®] capsules (NDA 50-708) and Injection (NDA 50-709) for the prophylaxis of organ rejection in patients receiving allogenic liver transplants. Supplemental NDAs 50-708 (S-008)/50-709 (S-009) were approved in April 1997 for prophylaxis of rejection after allogenic kidney transplantation.

A topical formulation of tacrolimus, Protopic[®] (tacrolimus) ointment, has been developed by Fujisawa for dermatologic use. Protopic[®] ointment is indicated for the treatment of atopic dermatitis. The rationale for this is that atopic dermatitis is considered an immunologic disorder believed to be modified by T-lymphocytes. The sponsor would like to market two strengths of the Protopic[®] ointment (0.03% and 0.1%).

The nonclinical pharmacology/toxicology of orally or intravenously administered tacrolimus has been established under NDAs 50-708/50-709. Oral carcinogenicity studies were

conducted in mice and rats for tacrolimus. The following information was included in the Prograf[®] label concerning potential carcinogenicity of tacrolimus.

“An increased incidence of malignancy is a recognized complication of immunosuppression in recipients of organ transplants. The most common forms of neoplasm are non-Hodgkin's lymphomas and carcinomas of the skin. As with other immunosuppressive therapies, the risk of malignancies in Prograf recipients may be higher than in the normal, healthy population. Lymphoproliferative disorders associated with Epstein-Barr Virus infection have been seen. It has been reported that reduction or discontinuation of immunosuppression may cause the lesions to regress.”

“Carcinogenicity studies were carried out in male and female rats and mice. In the 80-week mouse study and in the 104-week rat study no relationship of tumor incidence to tacrolimus dosage was found. The highest doses used in the mouse and rat studies were 0.8-2.5 times (mice) and 3.5-7.1 times (rats) the recommended clinical dose range of 0.1-0.2 mg/kg/day when corrected for body surface area.”

Reviewer Comments: It is important to note that the pharmacokinetic data available from the systemic (oral-feed) carcinogenicity studies referred to in the Prograf label indicated that the bioavailability of tacrolimus from the feed was very poor. This may be a contributing factor for why no tumors were noted in either the rat or mouse systemic carcinogenicity studies. This will be discussed in more detail in the evaluation of tumor findings section located near the end of this document.

The sponsor submitted IND _____ to the division in December 1994 for studying the efficacy and safety of Protopic[®] ointment in the treatment of atopic dermatitis. Additional nonclinical pharmacology/toxicology studies were conducted with the Protopic[®] ointment under IND _____ to support the safety of topical application of Protopic[®] ointment. The focus of this review is to evaluate the dermal carcinogenicity study conducted with tacrolimus ointment. The rationale for conducting a review of the dermal carcinogenicity study separately is to provide the data to the Executive Carcinogenicity Assessment Committee for evaluation. The rest of the nonclinical pharmacology/toxicology studies conducted to support tacrolimus ointment will be provided in a subsequent review.

The study report for the dermal carcinogenicity study was originally submitted to IND _____ (Serial No. 117; 2-5-99). The dermal carcinogenicity study was resubmitted to IND _____ (Serial No. 124; 5-13-99) with the requested SAS datasets for statistical evaluation. No formal review was conducted for this study under the IND. A formal review was conducted under the NDA submission. Tom Hammerstrom performed a statistical consult review for the dermal carcinogenicity study. The conclusions from his review will be provided below.

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Studies reviewed within this submission:

(Note: Only one study is reviewed in this addendum review. The rest of the nonclinical pharmacology/toxicology studies are reviewed in the original review.)

- 1) Topical oncogenicity study of FR900506 (FK506, tacrolimus) ointment in B6C3F1 mice following daily administration for 24 months

CARCINOGENICITY:

Study Title: Topical oncogenicity study of FR900506 (FK506, tacrolimus) ointment in B6C3F1 mice following daily administration for 24 months

Study Number: 95-8005

Volume Numbers: 27 – 39

Test Facility: _____

Study Date(s): 1-24-96 to 1-30-98

Date of Submission: 9-9-99

GLP Compliance/Quality Assurance: Yes

QA- Report: Yes (X) No ()

Study Type: Two year dermal carcinogenicity study in mice

Species/strain: B6C3F1 mice

Number of animals per group; age at start of study: 50 mice/sex/group for oncogenicity assessment and 60 mice/sex/group for toxicokinetic assessment; 4 weeks old (males: 7.7 – 22.4 g; females: 9.2 – 19.3 g)

Animal housing: The mice were individually housed in suspended stainless steel cages with wire mesh front and bottom.

Drug Lot/Batch number(s): refer to table below from electronic NDA submission

Drug Purity / Stability / Homogeneity: refer to table below from electronic NDA submission

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Group 1 animals received no application of test article but were subjected to all handling procedures. FR900506 ointment and vehicle (100 µl/mouse) were applied daily, seven days per week, for up to 105 consecutive weeks to an area equal to 40% of the estimated total body surface. The ointment from each application remained on the skin until the next application. The FR900506 ointment or vehicle control was applied to the test area by syringe at 2 µl/cm² and spread evenly with a rod. The treatment area was unabraded and unoccluded. Prior to the application of each dose, residual test article wiped off with gauze moistened with water and the test area was blotted dry. The treatment area was clipped free of hair on an as needed basis.

- *Basis of Dose Selection:* The FR900506 ointment concentrations in this study encompass 1X, 3X and 10X the ointment concentration dose range that were used in clinical studies.
- *Relation to Clinical Use:* The intended route in humans is topical administration.
- *CAC Concurrence:* No CAC concurrence was obtained for the doses selected in this study. The sponsor anticipated that this dose range would include the MTD for the study.
- *Restriction Paradigm for Dietary Restriction Studies:* NA
- *Route of Administration:* Topical
- *Frequency of Drug Administration:* 1X/day
- *Dual Controls Employed:* No
- *Interim Sacrifices:* No
- *Satellite PK or Special Study Group(s):* refer to study design table above
- *Unscheduled Sacrifices or Deaths:* Yes, will be discussed in mortality section below.

- *Deviations from Original Study Protocol:*

Animals were dying prior to the completion of the study in the higher dose groups. The sponsor informed the division when they first noticed that this was happening in the higher dose groups. The division advised the sponsor to stop dosing animals for a group when the number of surviving animals was 20/sex. The sponsor was advised to continue those animals until the end of the study period. If they survived until the end of the study after dosing was stopped, then it was recommended to perform a full analysis for those animals and note in the results when dosing was stopped for those animals.

Study Results and Frequency of Monitoring:

- *Clinical Observations:* Clinical observations were performed and recorded every four weeks for general health, physical appearance, behavior and pharmacologic or toxic effects other than those noted during observation of the skin. In addition, changes in behavior, reaction to treatment or general health were recorded for each animal.

A dose related occurrence of several abnormal clinical observations was noted in both males and females in groups 4-7. The dose related abnormal observations included cold to touch, hunched posture, lethargy, emaciated/thin and swelling. The noted clinical observations were due to high systemic levels of FR900506 obtained after topical application of FR900506 (refer to toxicokinetic section below for additional details).

- *Dermal Observations:* The characteristics of the skin (including palpation data) were recorded prior to treatment on Day 1 and weekly thereafter for signs of erythema, scaling, edema, tumors or other indications of skin toxicity. The locations, sizes, and progression history of tumors were recorded using a grid location system. Time to first tumor was recorded. Tumors were classified according to their gross visual appearance as papilloma, carcinoma, or unknown. Skin thickness (mm) was measured (main group animals only) every four weeks until the end of the study.

No evidence of erythema or edema was noted in any of the test article treated or vehicle treated groups during the study. Papillomas were present on the application site of two females in group 4. One was present from week 64 to 94 (animal sacrificed moribund at week 94) and one was present from week 66 to 67 (papilloma was no longer present at week 68). A papilloma was present on one female in group 5 from week 60 to terminal sacrifice.

Skin thickness was slightly increased for males and females in groups 3 and 4 compared to untreated control animals (from week 16 through week 104). Skin thickness was slightly increased for males and females in groups 5, 6 and 7 compared to untreated control animals (from week 16 through week 76 or until the death of all animals in a group). Skin thickness was slightly increased for group 2 males and females compared to untreated control animals for a few scattered number of measurements.

- *Mortality:* Animals were observed and viability was recorded twice daily. Animals judged to be in such condition that their survival was in question were euthanized and necropsied. Animals bearing a lesion grossly diagnosed as a life-threatening tumor, which had persisted for four weeks and had reached a diameter of 2 cm or more, were euthanized and the tumor taken for preservation and evaluation.

Animals in all groups died during the course of the study (refer to survival figures below taken directly from the electronic NDA submission). Mortality and moribund euthansia increased in both males and females with increasing dose level. All animals in Group 7 died during weeks 28 (males) and 29 (females). All animals in group 6 died in weeks 37 (males) and 47 (females). In group 5, 7/50 males and 8/50 females survived until terminal necropsy. Treatment was discontinued for Group 5 males and females 39 and 26 weeks, respectively, before terminal necropsy. Survival to terminal necropsy in the remaining groups was: 21/50 males and 20/50 females in group 4; 39/50 males and 39/50 females in group 3; 41/50 males and 44/50 females in group 2; and 42/50 males and 41/50 females in group 1. The sponsor reported that no tumor

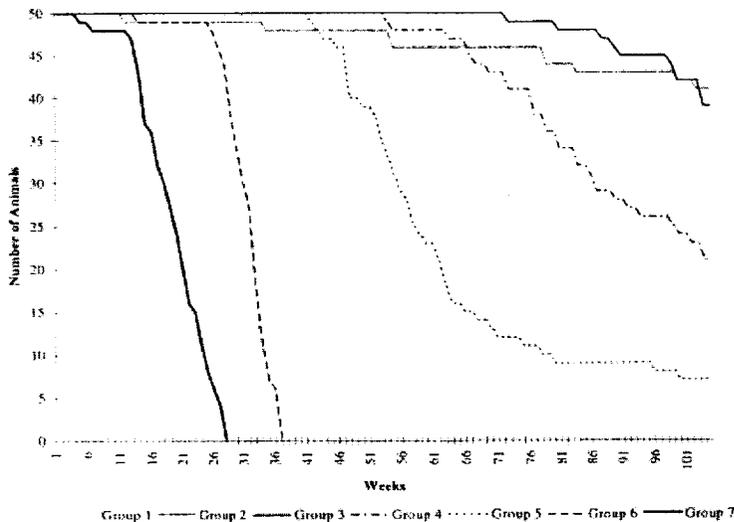
incidence was noted in the three highest dose groups. The animals died for no apparent reason. I suspect that the increased lethality in the three highest dose groups was due to overt toxicity related to high systemic levels of tacrolimus (refer to toxicokinetic section below).

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Page 55

FIGURE 1
SURVIVAL - MALES

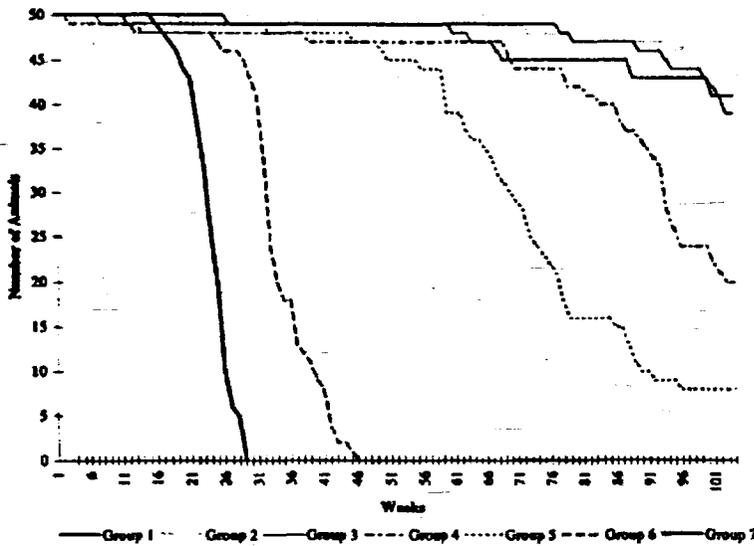


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Page 56

FIGURE 2
SURVIVAL - FEMALES



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- *Body Weight:* Body weights were recorded for each animal prior to treatment on Day 1, weekly for weeks 1-13, once every four weeks thereafter, and on the day before terminal euthanasia.

Body weight data for males and females is presented in the two figures below taken directly from the electronic NDA. Body weights in group 5, 6 and 7 males were significantly decreased starting with week 1 and for 50, 63 and 44% of the weeks, respectively, for which body weights were determined up to the death of all animals or up to week 104. Body weight for group 4 males was significantly decreased starting on week 9 and was significantly decreased for 28% of the weeks on which body weights were determined to week 104.

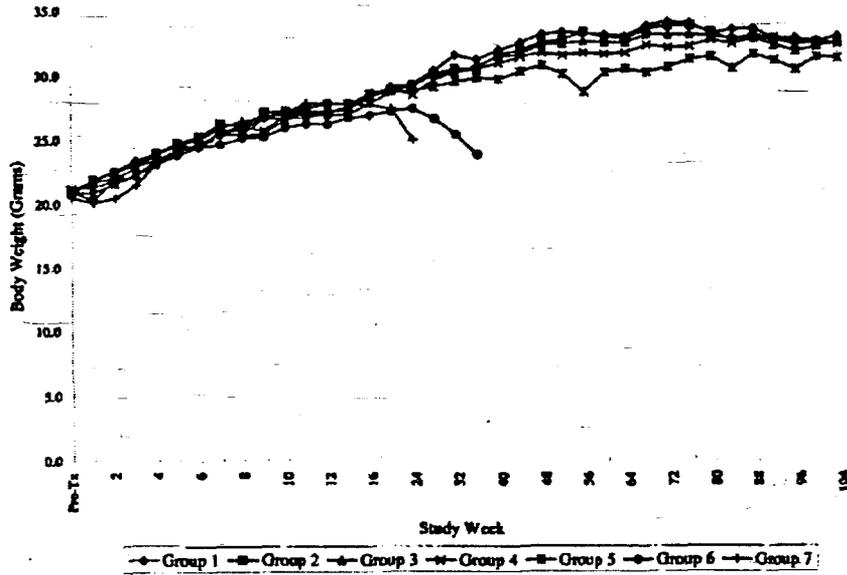
Body weights in group 5, 6 and 7 females were significantly decreased starting with week 1. However, the degree of body weight decrease was not as great as noted in male animals. Body weights were decreased for 6, 40 and 13% of the weeks, respectively, on which body weights were determined to the death of all animals in the group or to week 104.

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Study Number: 3-A20

Page 57

FIGURE 3
MEAN BODY WEIGHTS - MALES

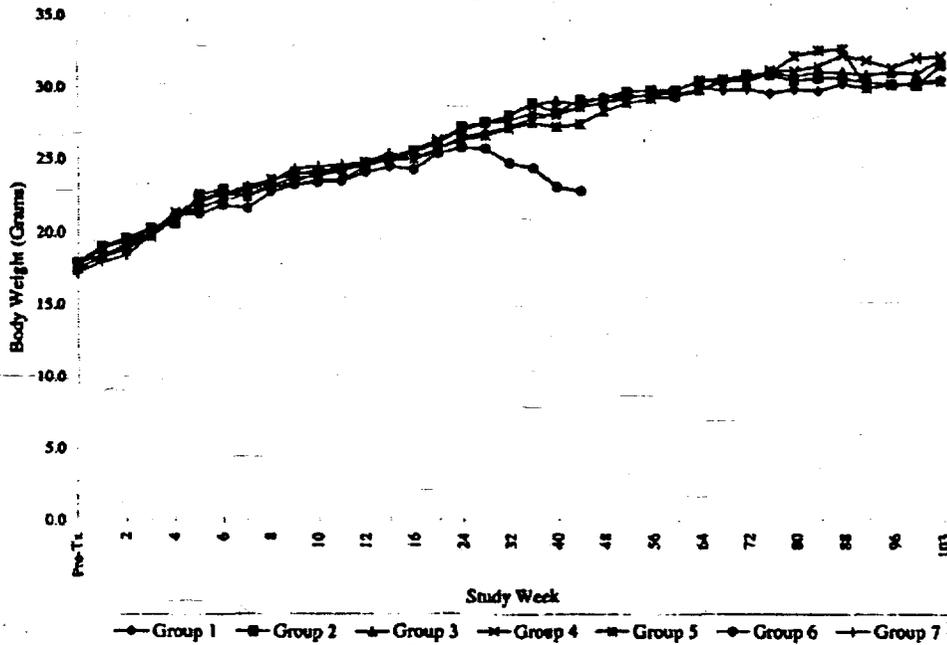


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Study Number: 3-A20

Page 58

FIGURE 4
MEAN BODY WEIGHTS - FEMALES



- *Food Consumption:* Individual food consumption was measured and recorded weekly for weeks 1-13 and once every four weeks thereafter.

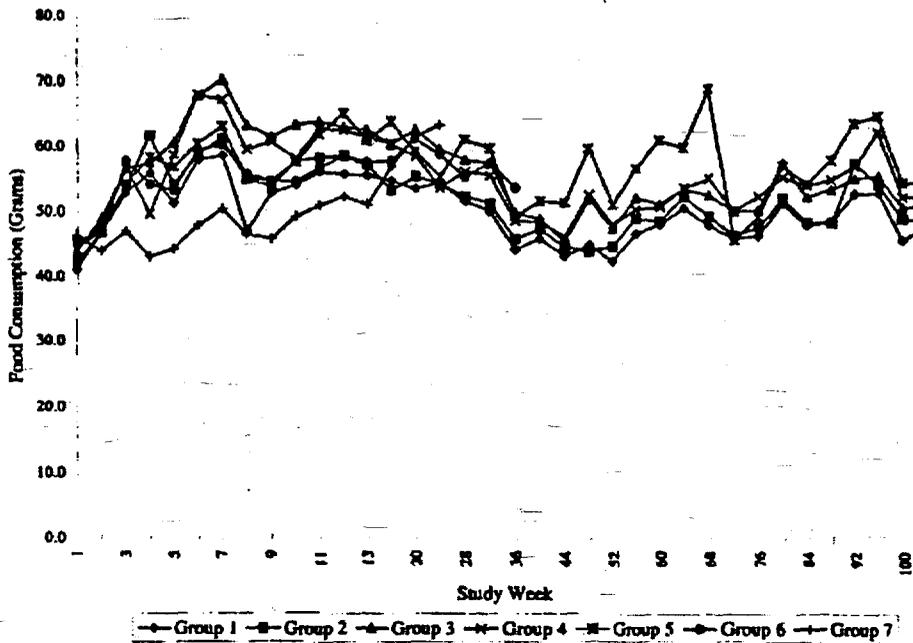
Food consumption data for males and females is presented in the two figures below taken directly from the electronic NDA. Food consumption did not consistently increase or decrease over the study. Increases and decreases in food consumption were noted over the study period for all groups as seen in the two figures below.

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Study Number: 3-A20

Page 59

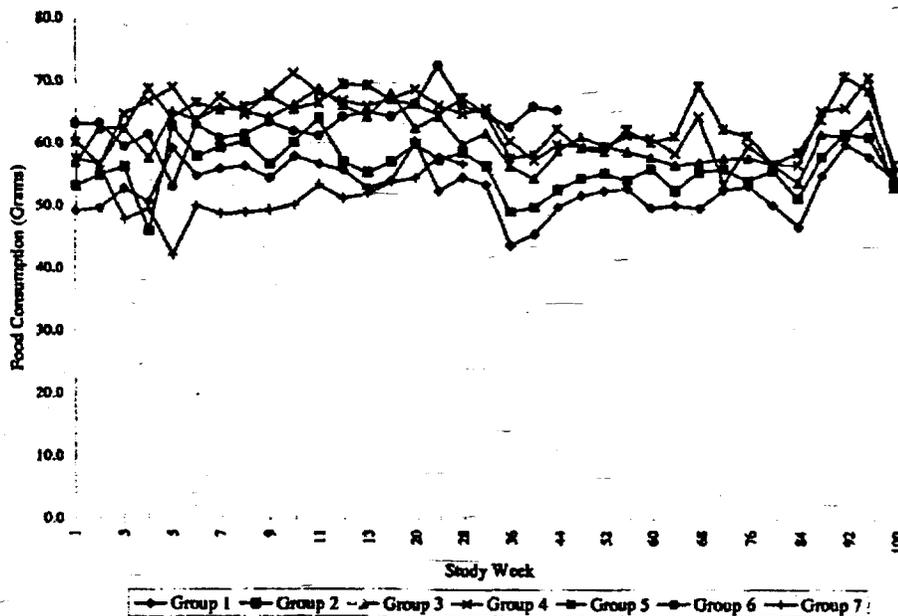
FIGURE 5
MEAN FOOD CONSUMPTION - MALES



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Study Number: 3-A20

Page 60

FIGURE 6
MEAN FOOD CONSUMPTION - FEMALES

- *Hematology*: Blood samples for hematology analysis were collected from any animal euthanized due to moribund condition and from all other animals at terminal euthanasia.

No treatment related effects on hematology parameters were noted in this study.

- *Organ Weights*: Adrenal glands, heart, kidneys, liver, ovaries, spleen and testes were weighed prior to fixation for all animals euthanized at the end of the study.

A few dose related differences in organ weights were noted in the study (refer to tables below). Dose dependent increases in liver and spleen weights were noted for male and female animals. Dose dependent decreases in kidney and heart weights were noted in male animals only. No differences in the incidence of neoplasia were noted that correlated with these trends.

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Mean organ weight values (grams) with dose related trends in males

Treatment	Number of Animals	Heart weight (↓)	Kidney Weight (↓)	Liver Weight (↑)	Spleen Weight (↑)
Sham Control	42	0.252	0.704	1.537	0.087
Vehicle Control	41	0.255	0.693	1.593	0.087
0.03% Tacrolimus	39	0.241	0.674	1.667	0.140
0.1% Tacrolimus	21	0.211	0.639	1.768	0.209

Mean organ weight values (grams) with dose-related trends in females

Treatment	Number of Animals	Liver Weight (↑)	Spleen Weight (↑)
Sham Control	41	1.883	0.315
Vehicle Control	44	1.713	0.189
0.03% Tacrolimus	39	1.894	0.240
0.1% Tacrolimus	20	2.326	0.425

Gross Pathology: Performed at necropsy.

Macroscopic observations typical of long term studies in mice were noted in this study. Nodules or masses in the skin were rarely noted in the skin. These correlated with a variety of neoplastic and nonneoplastic alterations which occurred with low frequency in treated and untreated skin. Common observations included nodules and masses in the liver, enlarged lymph nodes and spleens, kyphosis of the thoracolumbar spine, and thickening of, or cysts in the uterus. Distention of large and small bowel was observed in many decedent animals from groups 5-7, but was far less frequent in groups 1-4. This finding was most likely an artifact related to post mortem gas production rather than an effect of treatment. Discolorations, enlargements and reductions in size occurred in a variety of tissues without consistent patterns relating to treatment. The incidence of lymph node and splenic enlargement exhibited a treatment related response which correlated with the incidence of lymphoma noted in group 4 animals of both sexes.

- Histopathology: The following tissues were examined, collected for preservation at necropsy and examined histopathologically: Adrenal glands, aorta, bone marrow (femoral), brain (brain stem, cerebellum and cerebral cortex), cervix/vagina, epididymides, esophagus, eyes, femur, gallbladder, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, ileum, jejunum), kidneys, larynx/pharynx, liver, lungs, lymph nodes (mandibular, mesenteric), mammary gland (inguinal), ovaries, pancreas, pituitary gland, prostate gland, salivary glands (mandibular), sciatic nerve, seminal vesicles, skeletal muscle (thigh), skin (treated and untreated), spinal cord (thoracolumbar),

spleen, sternum, stomach, testes, thymus, thyroid/parathyroid glands, tongue, trachea, urinary bladder, uterus and gross lesions.

Note: Due to the level of mortality in groups 5, 6 and 7, no histopathological examination was conducted on these animals. The sponsor stated that the high mortality in these groups disqualified them from evaluation of carcinogenicity.

Non-Tumor findings:

A summary of the nonneoplastic findings, which occurred more frequently in test article, treated groups than in control groups is presented in the following table (obtained directly from electronic NDA submission). Tissues with dose-related increases in the incidence of histomorphologic changes included the heart, bone marrow, administration site skin and lymphoid tissues of both sexes.

Reviewer's Comments: It is important to note that in order to obtain a total number for each column the number outside of the parentheses and the one inside the parentheses should be added together. For example, the total number of male mice in group 4 is 21 (survived to terminal necropsy) + 29 (died or euthanized prior to terminal sacrifice) = 50 male mice. The total incidence for increased myelopoiesis in the femur marrow for group 4 male mice would be 5 + 13 = 18.

Text Table 13
Incidence of Selected Nonneoplastic Findings
Males

Group Number	1	2	3	4
Number of Animals	42 (8)	41 (9)	39 (11)	21 (29)
Tissue/Lesion				
Heart				
-inflammatory cell infiltrates	5	1 (3)	2 (2)	9 (8)
-myofiber degeneration	2 (2)	5 (2)	2 (3)	4 (9)
-myocardial fibrosis	2	3	9 (2)	11 (3)
-thrombosis				(3)
-vegetative bacterial endocarditis				(3)
Femur, marrow				
-increased myelopoiesis	6	5 (3)	4 (4)	5 (13)
Sternum, marrow				
-increased myelopoiesis	8	9 (3)	5 (3)	5 (11)
Administration site skin				
-acanthosis	2		10	10 (14)
-inflammatory cell infiltrates	2	1	12	9 (15)

Numbers in parentheses refer to animals which died prior to study termination

Text Table 13 (Concluded)
Incidence of Selected Nonneoplastic Findings
Females

Group Number	1	2	3	4
Number of Animals	41(9)	44(6)	39(11)	20(30)
Tissue/Lesion				
Heart				
-inflammatory cell infiltrates	4	1 (1)	3 (5)	5 (6)
-myofiber degeneration	1	2	1 (2)	1 (7)
-myocardial fibrosis	12	7	8 (3)	13(2)
-thrombosis	1			(1)
-vegetative bacterial endocarditis				(1)
Femur, marrow				
-increased myelopoiesis	5 (1)	2 (3)	3 (4)	7 (15)
Spleen				
-increased extramedullary hematopoiesis	7 (6)	4 (2)	7 (3)	8 (18)
Sternum, marrow				
-increased myelopoiesis	10(2)	2 (3)	2 (5)	8 (19)
Administration Site skin				
-acanthosis	1	8	22 (4)	11 (8)
-inflammatory cell infiltrates	8 (2)	12 (3)	27 (7)	17 (17)

Numbers in parentheses refer to animals which died prior to study termination

Nonneoplastic changes in the administration site skin, which had an increased incidence in treated animals, included acanthosis (hyperplasia of the epidermis) and increased lymphohistiocytic inflammatory cell infiltrates in the dermis.

Thymic involution/atrophy was present to some extent in most animals. However, dose related increases in the average severity grade were noted in group 3 and 4 animals. In addition, a dose dependent decrease in the numbers of extranodal lymphoid aggregates was noted in group 3 and 4 animals. A dose related increase in the incidence of increased myelopoiesis in the femoral and sternal marrow was noted in both sexes and increased extramedullary hematopoiesis in the spleens was noted in female mice. Increased myelopoiesis may be related to generalized stress and debility rather than to the test article itself since it was more prevalent in animals which died early.

Dose related changes in the heart included inflammation, myofiber degeneration and fibrosis. Three group 4 males and one group 4 female (all of which died early) had vegetative bacterial endocarditis. This may represent a complication of the pharmacologic action of the drug (i.e., immunosuppression).

Tumor findings:

Note: The sponsor's incidence of neoplastic histopathology findings is provided below in the addendum section. This table was copied directly from the electronic NDA. Thomas Hammerstrom performed the agency statistical analysis of the tumor findings. The results of this analysis will be summarized below. A copy of the statistical review is attached to this review as an addendum.

Cutaneous neoplasms were rare in both treated and untreated skin. Incidence of cutaneous neoplasms in treated and untreated skin sites combined is summarized below in a table obtained from the electronic NDA.

Study Number: 3-A20

Page 1150

Incidence of Cutaneous Neoplasms

Group Number	1	2	3	4
Number of Animals	42 (8) / 41 (9)	41 (9) / 44 (6)	39 (11) / 39 (11)	21 (29) / 20 (30)
Tissue/Lesion Sex	M / F	M / F	M / F	M / F
Skin, non-administration site -sebaceous adenoma		/ 1		
Skin, administration site -basal cell carcinoma -hemangioma -hemangiosarcoma -sarcoma, NOS -squamous cell carcinoma		(1) /	/ (1) / 1	/ (1) 1 /

Numbers in parentheses refer to animals which died prior to study termination

The CDER biostatistical reviewer independently performed analyses on the survival and tumor data. The biostatistical reviewer agreed with the sponsor that the fourth highest dose group was the MTD and excluded the three highest dose groups from subsequent analyses.

The biostatistical reviewer's preliminary analysis of fatal tumors found a highly statistically significant increase in hemolymphoretic tumors with increase in dose. The Cochran-Armitage trend test was statistically significant at level <0.001 in both sexes. This result was in contrast to the results of the Sponsor's analysis. The sponsor performed a Peto analysis for malignant neoplasms which revealed a significant difference in male mice for the hemolymphoretic system, but not in female mice (males p=0.0129, females p=0.6415). The analysis performed by the agency's biostatistical reviewer will be the one that will be relied upon

for this review. The results of the agency's statistical analysis will be discussed following the table of neoplastic findings provided below.

A complete copy of the summary neoplastic lesions data is provided as an addendum to this review below. This information was copied directly from the electronic NDA submission.

Tumor rate incidence table based on the summary tables for animals that underwent scheduled and unscheduled sacrifices is provided below.

**Tumors Observed in Animals from Scheduled and Unscheduled Sacrifices
(Including Animals Found Dead)**

Note: Neoplastic incidences from the scheduled sacrifice are listed as the first numbers for each tumor type. Neoplastic incidences from unscheduled sacrifices or animals found dead are listed as the second numbers for each tumor type. Total values for scheduled and unscheduled tumor incidences are in parentheses as the third number for each tumor type. Numbers in **bold** indicate possible effects that the CDER biostatistical reviewer was asked to confirm.

	Males				Females			
	Untreated	Vehicle	0.03%	0.1%	Untreated	Vehicle	0.03%	0.1%
<i>Lungs</i>								
Adenoma, bronchiolo-	7/42	8/41	8/39	4/21	3/41	2/44	5/39	2/20
(Total)	1/8 (8/50)	0/9 (8/50)	2/11 (10/50)	3/29 (7/50)	0/9 (3/50)	1/6 (3/50)	2/11 (7/49)	0/30 (2/50)
Carcinoma, metastati	0/42	0/41	0/39	1/21				
(Total)	1/8 (1/50)	0/9 (0/50)	0/11 (0/50)	0/29 (1/50)				
Pheochromocytoma					1/41	0/44	0/39	0/20
(Total)					0/9 (1/50)	0/6 (0/50)	0/11 (0/49)	0/30 (0/50)
Hemangiosarcoma					0/41	0/44	0/39	0/20
(Total)					1/9 (1/50)	0/6 (0/50)	0/11 (0/49)	0/30 (0/50)

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<i>Heart</i>								
Hemangiosarcoma	1/42 0/8 (1/50)	0/41 0/9 (0/50)	0/39 0/11 (0/50)	0/21 0/29 (0/50)				
<i>Liver</i>								
Adenoma	8/42 2/8 (10/50)	12/41 0/9 (12/50)	7/39 2/11 (9/50)	6/21 6/29 (12/50)	2/41 0/9 (2/50)	6/44 0/6 (6/50)	2/39 3/11 (5/49)	5/20 3/30 (8/50)
(Total)								
Carcinoma	1/42 4/8 (5/50)	5/41 1/9 (6/50)	11/39 3/11 (14/50)	3/21 1/29 (4/50)	3/41 0/9 (3/50)	3/44 1/6 (4/50)	1/39 0/11 (1/49)	2/20 0/30 (2/50)
(Total)								
Combined Adenoma + Carcinoma	9/42 6/8 (15/50)	17/41 1/9 (18/50)	18/39 5/11 (23/50)	9/21 7/29 (16/50)	5/41 0/9 (5/50)	9/44 1/6 (10/50)	3/39 3/11 (6/49)	7/20 3/30 (10/50)
(Total)								
Hemangioma					1/41 0/9 (1/50)	0/44 0/6 (0/50)	0/39 0/11 (0/49)	0/20 0/30 (0/50)
(Total)								
Hemangiosarcoma	2/42 1/8 (3/50)	2/41 0/9 (2/50)	1/39 1/11 (2/50)	0/21 4/29 (5/50)	1/41 2/9 (3/50)	2/44 2/6 (4/50)	0/39 0/11 (0/49)	0/20 0/30 (0/50)
(Total)								
Hepatoblastoma					0/41 0/9 (0/50)	0/44 0/6 (0/50)	0/39 1/11 (1/49)	0/20 0/30 (0/50)
(Total)								
<i>Kidneys</i>								
Adenoma, Tubular Epi (Total)					0/41 0/9 (0/50)	1/44 0/6 (1/50)	0/39 0/11 (0/50)	0/20 0/30 (0/50)
<i>Skin (Untreated)</i>								
Sebaceous Adenoma (Total)					0/40 0/9 (0/49)	1/43 0/6 (1/49)	0/39 0/11 (0/50)	0/20 0/30 (0/50)
<i>Thymus</i>								
Thymoma (Total)					0/40 0/9 (0/49)	0/44 0/6 (0/50)	1/38 0/11 (1/49)	0/17 0/30 (0/47)
<i>Pancreas</i>								
Adenoma, Islet Cell (Total)	0/42 0/8 (0/50)	1/41 0/9 (1/50)	0/39 0/10 (0/49)	0/21 0/29 (0/50)	1/41 0/9 (1/50)	0/44 0/6 (0/50)	0/39 0/11 (0/50)	0/20 0/30 (0/50)
Neurofibroma (Total)	0/42 0/8 (0/50)	0/41 0/9 (0/50)	0/39 0/10 (0/49)	0/21 1/28 (1/50)				
<i>Salivary Glands</i>								
Hemangioma (Total)	0/42 0/8 (0/50)	0/41 0/9 (0/50)	0/39 0/11 (0/50)	1/21 0/29 (1/50)				
<i>Urinary Bladder</i>								
Hemangioma (Total)	1/42 0/8 (1/50)	0/41 0/9 (0/50)	0/39 0/11 (0/50)	0/21 0/29 (0/50)				

Hemangiosarcoma	0/42	0/41	1/39	0/21				
(Total)	0/8 (0/50)	0/9 (0/50)	0/11 (1/50)	0/29 (0/50)				
<i>Duodenum</i>								
Adenoma, Papillary	0/42	0/41	0/39	0/21				
(Total)	0/6 (0/48)	0/7 (0/48)	1/7 (1/46)	0/23 (0/44)				
<i>Jejunum</i>								
Adenocarcinoma	1/42	0/41	0/38	0/21				
(Total)	0/8 (1/50)	0/9 (0/50)	0/11 (0/49)	0/29 (0/50)				
<i>Adrenal Glands</i>								
Spindle Cell Adenoma	0/42	0/41	1/39	0/21	0/41	0/44	0/38	0/20
(Total)	0/8 (0/50)	0/9 (0/50)	0/11 (1/50)	0/29 (0/50)	1/9 (1/50)	0/6 (0/50)	0/11 (0/49)	0/30 (0/50)
Pheochromocytoma					1/41	0/44	0/38	1/20
(Total)					0/9 (1/50)	0/6 (0/50)	0/11 (0/49)	0/30 (1/50)
<i>Mammary Gland</i>								
Liposarcoma					0/41	0/44	0/39	0/20
(Total)					1/9 (1/50)	0/6 (0/50)	0/11 (0/50)	0/30 (0/50)
<i>Pituitary Gland</i>								
Adenoma					7/40	4/43	4/39	0/18
(Total)					0/9 (7/49)	0/6 (4/49)	0/11 (4/50)	0/30 (0/48)
Carcinoma					0/40	0/43	0/39	1/18
(Total)					0/9 (0/49)	0/6 (0/49)	0/11 (0/50)	0/30 (1/48)
<i>Brain</i>								
Ependymoma					0/41	0/44	0/39	0/20
(Total)					1/9 (1/50)	0/6 (0/50)	0/11 (0/50)	0/30 (0/50)
<i>Stomach</i>								
Papilloma					0/41	0/44	0/39	0/20
(Total)					0/8 (0/49)	0/6 (0/50)	0/11 (0/50)	1/29 (1/49)
<i>Femur</i>								
Hemangiosarcoma	1/42	0/41	1/39	0/20	0/41	0/44	1/39	0/20
(Total)	0/8 (1/50)	1/9 (1/50)	0/11 (1/50)	0/28 (0/48)	0/9 (0/50)	1/6 (1/50)	0/11 (1/49)	0/30 (0/50)
<i>Testes</i>								
Carcinoma, Leydig	1/42	0/41	0/39	0/21				
(Total)	0/8 (1/50)	0/9 (0/50)	0/11 (0/50)	0/29 (0/50)				
<i>Epididymides</i>								
Leiomyosarcoma	0/42	1/41	0/39	0/21				
(Total)	0/8 (0/50)	1/9 (2/50)	0/11 (0/50)	0/29 (0/50)				
<i>Prostrate</i>								

Leiomyosarcoma	0/42	0/41	1/39	0/20				
(Total)	0/8 (0/50)	0/9 (0/50)	0/11 (1/50)	0/29 (0/49)				
<i>Ovaries</i>								
Cystadenoma					2/40	1/42	2/39	0/20
(Total)					1/9 (3/49)	0/6 (1/48)	0/9 (2/48)	1/30 (1/50)
Granulosa Cell Tumor					0/40	0/42	2/39	0/20
(Total)					0/9 (0/49)	0/6 (0/48)	0/9 (2/48)	0/30 (0/50)
Hemangioma					0/40	0/42	0/39	0/20
(Total)					0/9 (0/49)	0/6 (0/48)	0/9 (0/48)	1/30 (1/50)
Hemangiosarcoma					0/40	1/42	0/39	0/20
(Total)					1/9 (1/49)	1/6 (2/48)	0/9 (0/48)	0/30 (0/50)
<i>Cervix</i>								
Adenocarcinoma					1/40	0/42	1/36	0/19
(Total)					0/9 (1/49)	1/5 (1/47)	0/11 (1/47)	0/26 (0/45)
Fibroma					0/40	0/42	0/36	0/19
(Total)					0/9 (0/49)	0/5 (0/47)	0/11 (0/47)	1/26 (1/45)
Granular Cell Tumor					0/40	1/42	0/36	0/19
(Total)					0/9 (0/49)	0/5 (1/47)	0/11 (0/47)	0/26 (0/45)
Hemangiosarcoma					0/40	0/42	1/36	0/19
(Total)					0/9 (0/49)	0/5 (0/47)	0/11 (1/47)	0/26 (0/45)
Neurilemoma					0/40	1/42	0/36	0/19
(Total)					0/9 (0/49)	0/5 (1/47)	0/11 (0/47)	0/26 (0/45)
Polyp, Fibroepitheli					1/40	0/42	0/36	0/19
(Total)					0/9 (1/49)	0/5 (0/47)	0/11 (0/47)	0/26 (0/45)
Stromal Cell Sarcoma					0/40	0/42	4/36	0/19
(Total)					0/9 (0/49)	0/5 (0/47)	1/11 (5/47)	0/26 (0/45)
<i>Uterus</i>								
Adenocarcinoma					2/41	1/44	3/39	0/20
(Total)					0/9 (2/50)	1/6 (2/50)	0/11 (3/50)	0/30 (0/50)
Adenoma					0/41	0/44	1/39	0/20
(Total)					0/9 (0/50)	0/6 (0/50)	0/11 (1/50)	0/30 (0/50)
Fibroepithelial Poly					1/41	1/44	1/39	0/20
(Total)					0/9 (1/50)	0/6 (1/50)	0/11 (1/50)	0/30 (0/50)
Hemangiosarcoma					0/41	1/44	1/39	0/20
(Total)					0/9 (0/50)	0/6 (1/50)	0/11 (1/50)	0/30 (0/50)

Leiomyoma					0/41	0/44	2/39	0/20
(Total)					0/9 (0/50)	0/6 (0/50)	1/11 (3/50)	1/30 (1/50)
Myxosarcoma					0/41	0/44	1/39	0/20
(Total)					0/9 (0/50)	0/6 (0/50)	0/11 (1/50)	0/30 (0/50)
<i>Spleen</i>								
Hemangioma	0/42	0/41	0/39	1/21				
(Total)	0/8 (0/50)	0/9 (0/50)	0/11 (0/50)	0/29 (1/50)				
Hemangiosarcoma	1/42	1/41	2/39	0/21	0/41	0/44	1/39	0/20
(Total)	1/8 (2/50)	1/9 (2/50)	0/11 (2/50)	0/29 (0/50)	0/9 (0/50)	1/6 (1/50)	0/11 (1/50)	0/30 (0/50)
<i>Thyroid gland</i>								
Adenoma	0/42	0/41	1/39	0/21	0/41	0/44	1/38	1/20
(Total)	0/8 (0/50)	0/8 (0/49)	0/11 (1/50)	1/29 (1/50)	0/9 (0/50)	0/6 (0/50)	0/11 (1/49)	0/30 (1/50)
<i>Admin Site (Skin)</i>								
Carcinoma, Squamous	0/42	0/41	0/39	1/21				
(Total)	0/8 (0/50)	0/9 (0/50)	0/11 (0/50)	0/29 (0/50)				
Hemangioma					0/41	0/44	0/39	0/20
(Total)					0/9 (0/50)	0/6 (0/50)	0/11 (0/50)	1/30 (1/50)
Hemangiosarcoma	0/42	0/41	0/39	0/21				
(Total)	0/8 (0/50)	1/9 (1/50)	0/11 (0/50)	0/29 (0/50)				
Sarcoma, NOS					0/41	0/44	1/39	0/20
(Total)					0/9 (0/50)	0/6 (0/50)	0/11 (1/50)	0/30 (0/50)
Basal Cell Carcinoma					0/41	0/44	0/39	0/20
(Total)					0/9 (0/50)	0/6 (0/50)	1/11 (1/50)	0/30 (0/50)
<i>Sternum</i>								
Hemangiosarcoma	1/42	0/41	0/39	0/19				
(Total)	0/8 (1/50)	1/9 (1/50)	0/11 (0/50)	0/29 (0/40)				
Osteosarcoma					0/41	0/44	1/38	0/20
(Total)					0/9 (0/50)	0/6 (0/50)	0/11 (1/49)	0/30 (0/50)
<i>Skeletal Muscle</i>								
Hemangiosarcoma				1/1				
(Total)				(1/1)				
<i>Harderian Glands</i>								
Adenoma	1/1	3/3			2/2		1/1	
(Total)	(1/1)	(3/3)			(2/2)		(1/1)	
<i>Clitoral Glands</i>								
Hemangioma					0/1	1/2	0/1	
(Total)					(0/1)	(1/2)	(0/1)	
<i>Body Cavities</i>								

Sarcoma, NOS		1/2						
(Total)		(1/2)						
<i>Hemolymphoretic System</i>								
Histiocytic Sarcoma	0/42	0/41	0/39	2/21	3/41	0/44	0/39	1/20
(Total)	0/8 (0/50)	1/9 (1/50)	2/11 (2/50)	0/29 (2/50)	2/9 (5/50)	0/6 (0/50)	1/11 (1/50)	3/30 (4/50)
Lymphoma, Lymphocytic					1/41	1/44	0/39	0/20
(Total)					0/9 (1/50)	0/6 (1/50)	2/11 (2/50)	2/30 (2/50)
Lymphoma, Pleomorphic	6/42	2/41	3/39	14/21	11/41	5/44	12/39	17/20
(Total)	1/8 (7/50)	0/9 (2/50)	1/11 (4/50)	11/29 (25/50)	1/9 (12/50)	1/6 (6/50)	2/11 (14/50)	10/30 (27/50)
Lymphoma, Undifferentiated	0/42	1/41	0/39	0/21	2/41	1/44	1/39	1/20
(Total)	0/8 (0/50)	0/9 (1/50)	2/11 (2/50)	4/29 (4/50)	1/9 (3/50)	0/6 (1/50)	2/11 (3/50)	12/30 (13/50)
Plasmacytoma	0/42	0/41	0/39	1/21	0/41	0/44	0/39	1/20
(Total)	0/8 (0/50)	1/9 (1/50)	0/11 (0/50)	2/29 (3/50)	0/9 (0/50)	0/6 (0/50)	0/11 (0/50)	0/20 (1/50)

Statistically significant increases in pleomorphic lymphoma and in undifferentiated lymphoma were noted by the biostatistical reviewer. The Cochran-Armitage trend test showed that the incidence of pleomorphic lymphoma was statistically significant in female ($p < 0.0001$) and in male ($p < 0.0001$) animals. The Cochran-Armitage trend test showed that the incidence of undifferentiated lymphoma was statistically significant in female animals ($p = 0.0005$) but not in male animals ($p = 0.18$). The Cochran-Armitage trend test was run including vehicle, 0.03% and 0.1% dose groups.

The pairwise dose comparisons demonstrated that the carcinogenic effect was found only in the high dose group (0.1% tacrolimus ointment) compared to untreated control. The p-values for pleomorphic lymphomas for high dose animals compared to untreated control were 0.0009 and 0.0001 for both female and male animals, respectively. The p-values for undifferentiated lymphomas for high dose animals compared to untreated control were 0.004 and 0.05 for female and male animals, respectively.

Therefore, both the trend and pairwise comparison demonstrated that the incidence of pleomorphic lymphoma was statistically significant in male and female animals and that the incidence of undifferentiated lymphoma was statistically significant in female animals only.

The biostatistical reviewer generated Kaplan-Meier curves for the estimated times to fatal hemolymphoretic tumors for male and female animals. About half (30% of all animals) of the ~50% deaths by week 104 in the high dose group were due to fatal hemolymphoretic tumors in males. A larger portion of the high dose group deaths were due to fatal hemolymphoretic tumors in females. Approximately 55% of females in the high dose group died by week 104 and ~50% had fatal hemolymphoretic tumors. The p-values for the log-rank tests for a dose effect on time to fatal hemolymphoretic tumor were < 0.0001 for both sexes.

The results of the agency's biostatistical review contrast with the sponsor's statistical analysis. The sponsor asserts that a statistically significant increase in hemolymphoretic tumors was noted in males only. The agency's biostatistical review disagrees with this and notes that a statistically significant increase in hemolymphoretic tumors was noted in female and male animals.

The biostatistical reviewer informed me that no additional tumors noted in this study were statistically significant. This included liver adenomas and carcinomas and the combined value of liver adenomas + carcinomas. I requested that the biostatistical reviewer also analyze total hemangioma and hemangiosarcoma for all sites. No statistically significant difference was found for either hemangioma or hemangiosarcoma totals for all sites.

- *Toxicokinetics:* Blood samples were obtained from the satellite animals (4/sex/treated dose level/timepoint) at 4, 8 and 24 hours after application during weeks 1, 26, 52, 78 and 104. Wherever possible, toxicokinetic samples were collected from moribund euthansia animals. Toxicokinetic analysis was performed at _____ Blood concentrations of FR900506 were determined by _____

The sponsor notes that very large inter-individual differences in FR900506 concentrations were observed in this study. Blood concentrations of FR900506 (C_{max} and AUC_{0-24hr}) tended to increase with the concentration of FR900506 in the ointment. No difference in pharmacokinetic parameters was noted between male and female mice. No accumulation of FR900506 was noted over the study period. The toxicokinetic parameters are summarized in the following table.

Summary of Toxicokinetic Parameters

Sex	Conc.	Week 1		Month 6		Month 12		Month 18	
		C_{max}	AUC	C_{max}	AUC	C_{max}	AUC	C_{max}	AUC
Male	0.03%	11	163	9.0	169	6.8	97	7.8	114
	0.1%	27	421	32	490	27	275	31	373
	0.3%	34	504	60	868	48	464	nc	nc
	1%	60	859	156	2119	nc	nc	nc	nc
	3%	527	6813	987	12824	nc	nc	nc	nc
Female	0.03%	18	198	16	235	10	148	8.1	114
	0.1%	50	646	38	582	38	522	37	559
	0.3%	37	597	63	920	62	866	nc	nc
	1%	185	2187	157	2461	nc	nc	nc	nc
	3%	480	6531	860	11160	nc	nc	nc	nc

Conc.: FR900506 ointment concentration

C_{max} : ng/ml

AUC: $AUC_{0-24 hr}$ (ng·hr/ml)

nc: Not calculated due to high mortality in group

Overall Interpretation and Evaluation:*- Adequacy of the carcinogenicity studies and appropriateness of the test model:*

The mouse model is an appropriate model for analysis of dermal carcinogenicity. Even though a formal dose range study was not conducted for this dermal carcinogenicity study, the study reached an MTD based on mortality. The mortality was severe enough over the dose range tested that only two doses (0.03% and 0.1%) had adequate numbers of animals to assess the dermal carcinogenic potential of tacrolimus ointment. The MTD was identified as the 0.1% tacrolimus ointment dose based on mortality.

- Evaluation of Tumor Findings:

Both the trend and pairwise statistical comparison demonstrated that the incidence of pleomorphic lymphoma was statistically significant in male and female animals and that the incidence of undifferentiated lymphoma was statistically significant in female animals only. This is not a surprising finding based on the pharmacology of tacrolimus, an immunosuppressant agent. Significant systemic absorption was noted after topical administration of tacrolimus ointment. This could explain why lymphomas were noted in this dermal carcinogenicity study and not in the systemic administration carcinogenicity studies. My understanding of the systemic administration carcinogenicity studies is that there was a low level of systemic absorption after administration of tacrolimus via feed. This may explain why lymphomas were not observed in the systemic administration carcinogenicity studies.

Summary Conclusions and Recommendations:*- Acceptability of Study(s) or Overall Testing Approach:*

I believe that this study is acceptable, because an MTD was obtained in the study. The deaths observed in the three highest dose groups (0.3%, 1% and 3% tacrolimus ointment) were due to toxicity. Higher doses than 0.1% tacrolimus ointment could not be tolerated due to increased mortality. The overall testing approach to use the mouse for this dermal carcinogenicity study is appropriate.

- Major Tumor Findings:

The major tumor findings were pleomorphic lymphoma and undifferentiated lymphoma. Both the trend and pairwise statistical comparison demonstrated that the incidence of pleomorphic lymphoma was statistically significant in male and female animals and that the incidence of undifferentiated lymphoma was statistically significant in female animals only. The tumors were statistically significantly higher in the high dose group (0.1%) only. The no effect dose level for pleomorphic lymphoma and undifferentiated lymphoma is 0.03% tacrolimus ointment.

- *Non-neoplastic Findings:*

Tissues with dose-related increases in the incidence of histomorphologic changes included the heart, bone marrow, administration site skin and lymphoid tissues of both sexes.

Nonneoplastic changes in the administration site skin, which had an increased incidence in treated animals, included acanthosis (hyperplasia of the epidermis) and increased lymphohistiocytic inflammatory cell infiltrates in the dermis.

Thymic involution/atrophy was present to some extent in most animals. However, dose related increases in the average severity grade were noted in group 3 and 4 animals. In addition, a dose dependent decrease in the numbers of extranodal lymphoid aggregates was noted in group 3 and 4 animals. A dose related increase in the incidence of increased myelopoiesis in the femoral and sternal marrow was noted in both sexes and increased extramedullary hematopoiesis in the spleens was noted in female mice. Increased myelopoiesis may be related to generalized stress and debility rather than to the test article itself since it was more prevalent in animals which died early.

Dose related changes in the heart included inflammation, myofiber degeneration and fibrosis. Three group 4 males and one group 4 female (all of which died early) had vegetative bacterial endocarditis. This may represent a complication of the pharmacologic action of the drug (i.e., immunosuppression).

- *Biological Significance:*

The increased incidence of pleomorphic and undifferentiated lymphoma are biologically significant. These two types of lymphoma are probably due to the pharmacologic effect of tacrolimus (an immunosuppressant).

- *Potential Clinical Implications of Findings:*

It has been established in the literature and is stated in the tacrolimus label that an increased incidence of malignancy is a recognized complication of immunosuppression therapy. The most common forms of neoplasm are non-Hodgkin's lymphomas and carcinomas of the skin. It is interesting to note that no carcinomas of the skin were noted in this study even though the immunosuppressant was topically applied and there was significant systemic absorption after topical administration. One possible explanation for this observation is that mice in this dermal carcinogenicity study were not exposed to sunlight (or solar simulated light). The incidence of skin carcinoma in humans that have received immunosuppressant therapy is increased in sun exposed areas of the skin. In addition, human papilloma virus may play a role in human skin carcinoma. Immunosuppressant therapy would decrease the human body's ability to suppress the expression of human papilloma virus and thereby increase the potential for skin carcinoma formation via the human papilloma virus expression.

The highest proposed dose for tacrolimus ointment is the 0.1% concentration. This is the concentration level that was the MTD in the dermal carcinogenicity study and the concentration at which a statistical increased incidence of pleomorphic and undifferentiated lymphoma was noted. Human pharmacokinetic analysis in the target population (atopic dermatitis patients) have been conducted with 0.3% tacrolimus ointment (Study 94-0-008) with single and repeat doses. The design of this study is provided in the following table taken directly from the electronic NDA.

Table 6-13: Treatment Group Assignments and Tacrolimus Ointment Exposure (Study 94-0-008)

Treatment Group	Age Range (Years)	N	Location of Disease	Amount 0.3% Ointment per Application (g)	Area of Application (cm ²)	Tacrolimus Exposure per Application (mg)
Adult	A	6	Trunk/limbs	0.5	100	1.5
	B	7	Face	0.5	100	1.5
	C	6	Trunk/limbs	2.5	500	7.5
	D	6	Trunk/limbs	5.0	1000	15.0
	E	6	Trunk/limbs	15	5000	45.0
Pediatric	5-6†	4	Trunk/limbs	0.25	50‡	0.75
	7-11	4	Trunk/limbs	0.5	100	1.5

†Patients 3-6 years of age were allowed by the protocol, but no one <5 years of age enrolled.

‡For the first application, ointment was inadvertently applied over 100 cm² in two patients.

Source: References [5] and [6]

The highest dose group in this study was the Adult treatment group E. A 15 gram aliquot of 0.3% tacrolimus ointment (45 mg tacrolimus) was applied to a 5000 cm² area. The 0.3% tacrolimus ointment used in this study is 3X the concentration level that lymphomas were noted in the dermal carcinogenicity study (0.1%) and 10X the no effect concentration level (0.03%) identified in the dermal carcinogenicity study. In addition, the 0.3% concentration is 3X the highest concentration that will be marketed for tacrolimus ointment. Blood samples were obtained for pharmacokinetic analysis on Days 1 and 8 after dose application. The pharmacokinetic results from this study are provided in the following table taken directly for the electronic NDA submission.

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