concentrations ≥ 242 µg/ml and cell growth was reduced at concentrations ≥ 119 µg/ml in the presence of S9.

- Test Agent Stability: ASM 981 precipitated in the treatment medium at concentrations ≥ 1 mg/ml. Chemical analysis of dosing solutions used in the assay demonstrated a purity of — by HPLC. ASM 981 dosing solutions were stable over the dose range tested in this assay.
- Metabolic Activation System: Aroclor 1254 induced rat liver S-9 plus cofactors

- Controls:
  - Vehicle: DMSO
  - Negative Controls: N/A
  - Positive Controls: Ethyl methanesulphonate was used in the non-activated system (12.5 mM for 3 hour exposure). Cyclophosphamide was used in the activated system (15 µM for 3 hour exposure).
  - Comments: Appropriate positive controls were used in this study.

- Exposure Conditions:
  - Incubation and sampling times: Plated cells were incubated with test article with (3 hr exposure with 17 hour recovery) or without S9 activation (3 hr exposure with 17 hour recovery or 20 hr exposure). One hundred metaphases from each plate were analyzed for the following structural aberrations: Chromatid breaks (including deletions), isolocus breaks, chromosome breaks, all various forms of chromatid exchanges, dicentrics, tricentrics etc., ring chromosomes and interstitial deletions. Cells with more than 5 aberrations were recorded as multiple aberrant cells. The mitotic index was determined by counting 1000 cells from each plate and recording the number of metaphases. The mitotic index was expressed as a percentage of the control value.
  - Doses used in definitive study:

  First experiment: 0, 7, 15, 32, 70, 151, 325 and 700 µg/ml without S9 (3 hr treatment); 0, 7, 15, 32, 70, 151, 325 and 700 µg/ml with S9 (3 hr treatment)
  Second experiment: 0, 3, 7, 15, 32, 70, 151 and 325 µg/ml without S9 (20 hr treatment); 0, 15, 30, 61, 122, 247, 497 and 1000 µg/ml with S9 (3 hr treatment)
  - Study design: Followed ICH protocol

- Analysis:
  - No. replicates: 2/dose
  - Counting method: Manual

- Criteria for Positive Results: The test article was considered positive if one or more of the mutant frequencies in the treated groups was statistically significantly ($p<0.05$) larger than the corresponding solvent control value and there was a significant ($p<0.05$) dose relationship indicated by linear trend.

Summary of individual study findings:

- Study Validity: Solvent control mutant frequencies fell within established ranges. Positive control results were appropriate. Dose range selected for the definitive study was appropriate according to ICH guidelines.

- Study Outcome: The test article produced a negative response in the presence and absence of S-9 activation. All of the concentrations tested in this study exhibited a mutant frequency that was similar to the corresponding solvent control.

Genetic Toxicology Study 5:

Mouse bone marrow micronucleus test by the oral route

Study Title: Mouse bone marrow micronucleus test by the oral route

Key Findings: ASM 981 was negative in the mouse bone marrow micronucleus test.

Study No:	T-124/203-111
Laboratory Study No:	MK 35
Study Type:	Mouse bone marrow micronucleus test
Volume # and Page #:	47, 5-220
Conducting Laboratory:	Sandoz Pharma Ltd., Basle, Switzerland
Date of Study Initiation:	5/15/95
GLP Compliance:	Yes
QA- Reports:	Yes (X) No ( )
Drug Lot Number:	ASM 981 – Batch# X054 0495
Formulation/vehicle:	Aqueous solution containing 0.5% Poloxamer 188 and 3.5% hydroxypropylmethyl cellulose (Note: The stock oral ASM 981 solution was referred to as the solid dispersion and consisted of 20% ASM 981 (w/w) solid dispersion in 0.5% Poloxamer 188 and 3.5% hydoxypropylmethyl cellulose as a carrier in water.)

Methodology:

- Species: CD-1 mice; 8 weeks old; males: 31 – 38 g; females: 24 – 29 g
- Dose Selection Criteria:
  - Basis of dose selection: Toxicity was assessed by the presence of clinical signs at the following timepoints: the first 15 minutes after application, in intervals between 45 and 180 minutes on the first and second application day and the observations for clinical signs were continued after the second application until the fourth day.
  - Range finding studies: Eight mice (4 males and 4 females) were treated with 2 doses of 500 mg/kg ASM 981 (applied on day 1 and day 2 separated by a 24 hour interval). Toxicity was assessed for 4 days. No clinical signs of toxicity were noted at this dose. The study report stated that the 500 mg/kg dose was the maximum feasible dose that could be administered with the ASM 981 "solid dispersion". One part "solid dispersion" with 9 parts of water was used

for the final dosing solution and 25 ml/kg was administered to achieve a 500 mg/kg dose level.

- Test Agent Stability: The purity of ASM 981 "solid dispersion" was ——— ASM 981 "solid dispersion" was dissolved in water and stored for 25 hour at 25°C. The reconstituted dispersion was stable for at least 24 hours if protected from direct light exposure.
- Metabolic Activation System: N/A

- Controls:
  - Vehicle: Aqueous solution containing 0.5% Poloxamer 188 and 3.5% hydroxypropylmethyl cellulose
  - Negative Controls: N/A
  - Positive Controls: Triethylenemelamine (TEM: 0.6 mg/kg).
  - Comments: An appropriate positive control was used in this study.

- Exposure Conditions:
  - Incubation and sampling times: Mice were treated with two doses (applied on day 1 and day 2 separated by a 24 hour interval) of 0, 50, 160 and 500 mg/kg ASM 981 or a single dose of 0.6 mg/kg TEM. Bone marrow for analysis of nucleated cells was obtained from treated mice 48 hours after the first dose.
  - Doses used in definitive study: discussed in previous section
  - Study design: Followed ICH protocol

- Analysis:
  - No. of animals: 5/sex/dose
  - Counting method: Stained bone marrow slides were analyzed by a ——— instrument for the presence of micronucleated polychromatic erythrocytes. For each mouse two slides were prepared and 2000 polychromatic erythrocytes per slide were analyzed for micronuclei. In the same scoring process, normochromatic erythrocytes were also analyzed separately for micronuclei.

- Criteria for Positive Results: The test article was classified as mutagenic if it induced a micronucleus frequency, which is statistically significantly above the control level. In addition, a dose response relationship is necessary for a positive response.

Summary of individual study findings:

- Study Validity: Solvent control mutant frequencies fell within established ranges. Positive control results were appropriate. Dose range selected for the definitive study was appropriate according to ICH guidelines.

- Study Outcome: No treatment related effect on the ratio of polychromatic to normochromatic erythrocytes was noted in this study. The mean percentages of micronucleated polychromatic erythrocytes (MPEs) were 0.17 ± 0.04%, 0.16 ± 0.03%, 0.20 ± 0.09% and 0.13 ± 0.05% for the control, low, mid and high ASM 981 dose levels, respectively. The mean percentage of MPEs was 2.62 ± 1.08% for the positive control group, which was significantly greater than the negative control value.

Genetic toxicology summary:

ASM 981 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.

Genetic toxicology conclusions:

ASM 981 did not demonstrate a positive genotoxicity signal based on the results of the *in vitro* and *in vivo* genotoxicity studies conducted for ASM 981.

CARCINOGENICITY:

Carcinogenicity Study #1:

SDZ ASM 981 cream: 52 week photocarcinogenesis study in hairless mice

Study Title: SDZ ASM 981 cream: 52 week photocarcinogenesis study in hairless mice
Study Number: T-128/BS-119

Note: The photocarcinogenicity study conducted with 1% ASM 981 cream was reviewed under IND [redacted] and will be summarized in this review.

The study design for the photocarcinogenicity study is provided in the following table (36/sex/group). The treatment regimen and UV exposure followed the established [redacted] protocol for photocarcinogenicity studies in Crl:SKH1-hr BR (albino hairless) mice.

Study Design

Treatment	Volume (µl/mouse/day)	Dose (mg/kg/day)	UVR Dose (MED)	UVR Dose (RBU/week)
Untreated Control	0	0	0.27	600
Vehicle Cream	30	0	0.27	600
0.2% ASM 981 Cream	30	2.4	0.27	600
0.6% ASM 981 Cream	30	7.2	0.27	600
1.0% ASM 981 Cream	30	12	0.27	600
Untreated Control	0	0	0.46	1200

ASM 981 cream or vehicle was applied to the back of each mouse by syringe and spread across the posterior dorsal skin to a total of 20% of the body surface area (~25 cm^2 area). There was no occlusion of the application site, nor were the mice fitted with neck collars. The UVR

source was a 6.5 kilowatt xenon long arc water cooled burner ——— The xenon arc was surrounded at a distance of 20 cm by a stationary octagonal metal frame holding one 15 x 15 cm glass filter ——— glass, 1 mm thick) on each facet to attenuate contribution in the UVC range. UVR intensity was monitored on all racks by a customized detector, which recorded both intensity and cumulative UVR dose (in ———).

The doses for this photocarcinogenicity study were selected on the basis of two previous studies conducted with the final market formulation of ASM 981 cream ——— Protocols 1014-001P and 1014-002). The first study was titled "8 week dermal range finding test in mice for photocarcinogenicity study" (Protocol 1014-001P). Doses of 0, 0.2, 0.6 and 1% ASM 981 cream (100 µl/day) were tested in the 8 week dose range finding study in mice. No signs of cutaneous phototoxicity or photoprotection were observed in this study. Mild to marked acanthosis/hyperkeratosis of the epidermis, hyperplasia of sebaceous glands and dermal inflammatory cell infiltrations were noted in all treated groups.

The second study was titled "4 week dermal pharmacokinetic study in mice for photocarcinogenicity study" (Protocol 1014-002). Doses of 0, 0.6% and 1% ASM 981 cream (30 or 50 µl/day) were tested in the 4 week dermal pharmacokinetic study in mice. The purpose of this study was to determine the level of systemic exposure in mice with these doses of the ASM 981 cream.

The mean toxicokinetic parameters obtained from this study are presented in the following table.

Treatment Group	C_{max} (ng/ml)	T_{max} (hr)	AUC_{0-24hr} (ng.hr/ml)
Male 0.6% cream, 50 µl	157	8	2150
Female 0.6% cream, 50 µl	178	8	2470
Male 1.0% cream, 50 µl	144	8	1950
Female 1.0% cream, 50 µl	355	8	4250
Male 0.6% cream, 30 µl	102	8	1360
Female 0.6% cream, 30 µl	134	8	1780
Male 1.0% cream, 30 µl	113	8	1630
Female 1.0% cream, 30 µl	200	8	2570

Peak blood concentrations were found at 8 hr post dose for all treatment groups. Female mice consistently demonstrated a higher systemic exposure to ASM 981 in all treatment groups. The contract lab explained that this result was due to the difference between male and female mice body weight. There appeared to be a dose dependent increase in C_{max} and AUC. ASM 981 levels could still be measured 24 hr post dose in all samples, ranging between ——— ng/ml.

Even though the results of the first dose ranging study suggested that the mouse could tolerate 100 µl of the 1% ASM 981 as the maximum dose, the sponsor selected to use a volume of 30 µl of the test article instead. The sponsor stated that the application volume of 30 µl per

animal was selected for the photocarcinogenicity study to avoid excessive systemic exposure at the high dose, which could lead to toxic effects in the lymphatic system. The concern was that this would complicate the interpretation of the results of the photocarcinogenicity study. This viewpoint was substantiated by the results of a 13 week dermal toxicity study conducted for ASM 981 (ethanol solution) in mice that was designed to investigate the threshold and time of onset for lymphoproliferative disorders. Thymus medullary atrophy (doses ≥50 mg/kg/day) and pleomorphic lymphoid proliferation (doses ≥100 mg/kg/day) was observed at 4 weeks. Thymus medullary atrophy (doses ≥25 mg/kg/day), pleomorphic lymphoid proliferation (doses ≥50 mg/kg/day) and pleomorphic lymphoma (doses ≥100 mg/kg/day) was observed at 8 weeks. Thymus medullary atrophy (doses ≥50 mg/kg/day) and pleomorphic lymphoma (doses ≥50 mg/kg/day) was observed at 13 weeks. Pleomorphic lymphomas occurred at mean $AUC_{(0-24\ hr)}$ of 6828 (M) and 8259 (F) ng•h/ml at 8 weeks and 5074 (M) and 3378 (F) ng•h/ml at 13 weeks.

The AUC values in the 4 week pharmacokinetic study conducted in mice with 50 µl of the final market formulation of ASM 981 cream were close to the AUC values at which pleomorphic lymphoma was noted at the 13 week timepoint in the 13 week dermal toxicity study. Therefore, it seemed reasonable to have selected 30 µl for each concentration of ASM 981 cream tested in the photocarcinogenicity study.

No treatment related effects on mortality, clinical signs, body weight or macroscopic findings were noted in this study. Topical administration of the vehicle increased the incidences of mice with cutaneous reactions (grade 2 erythema - both male and female mice; grade 2 flaking – male and female mice) compared to untreated low UVR control mice. All ASM 981 cream dose groups demonstrated a similar level of cutaneous reactions as was observed in the vehicle treated mice.

Topical administration of the vehicle enhanced photocarcinogenesis in this study. The enhancement of photocarcinogenesis tended to be greater in male mice, as compared to female mice. The median tumor onsets were 38.25 and 25.00 weeks in the male mice and 35.5 and 27.00 weeks in the female mice for untreated and vehicle treated animals, respectively. The reduction in median tumor onset periods was greater for the male mice (13.25 weeks) than for the female mice (8.5 weeks).

The median tumor onset for tumors ≥1 mm is provided in the following table.

ASM 981 Cream (%)	UVR Exposure —/Week)	Male Mice Median (Weeks)	Female Mice Median (Weeks)
None (Untreated)	600	38.25	35.50
0 (Vehicle)	600	25.00[c]	27.00[c]
0.2	600	27.00[c]	34.00[3]
0.6	600	25.00[c]	30.00[a,1]
1.0	600	26.00[c]	30.00[b,1]
None (Untreated)	1200	21.00[c,3]	20.00[c,3]

a = $p<0.05$ compared to untreated control; b = $p<0.01$ compared to untreated control; c = $p<0.001$ compared to untreated control.
1 = $p<0.05$ compared to vehicle control; 2 = $p<0.01$ compared to vehicle control; 3 = $p<0.001$ compared to vehicle control.

In this study, topical administration of the vehicle greatly enhanced photocarcinogenesis. The vehicle induced enhancement tended to be greater in male mice as compared to female mice. Topical administration of up to 30 µl of 1% ASM 981 cream had no influence on tumor development beyond the vehicle effect. In female mice, application of all doses of ASM 981 cream appeared to have a protective effect on the vehicle enhanced photocarcinogenesis. The reason for this is unclear. The vehicle enhancement of photocarcinogenesis has been noted in other photocarcinogenicity studies conducted in the literature and submitted to the agency. One potential explanation for this could be the modification of the optical quality of the skin with resulting enhancement of UVR penetration, which could lead to an increase in UVR induced skin tumors.

The photocarcinogenicity model used for this study is an appropriate animal model for analysis of photo co-carcinogenicity. The 4 week repeat dose pharmacokinetic study conducted in mice with ASM 981 cream supported use of 30 µl as the maximum volume of the 0.2%, 0.6% and 1.0% ASM 981 cream doses. However, a maximum tolerated dose (MTD) was not reached in this study. One of the premises stated in the 4 week pharmacokinetic study was that using 50 µl of each concentration of the ASM 981 cream would increase the possibility of lymphoma formation and potentially complicate the interpretation of the photocarcinogenicity aspect of this study. No indication of lymphoma was noted during the gross necropsy performed in this study. Therefore, it is unclear whether the 30 µl dose of the 1.0% ASM 981 cream could be interpreted as the MTD or not in this study.

One point to consider in this study is that the vehicle alone had a strong photocarcinogenic effect. It is unclear whether it would be possible to see any additional effect contributed by ASM 981 on top of this effect. Perhaps using the maximum feasible volume (100 µl) of the maximum feasible concentration (1% ASM 981 cream) would have been able to show some additional effect but perhaps not. This is one of the most difficult aspects of the design of the nonclinical photocarcinogenicity study. When the vehicle alone has such a drastic effect, it

becomes difficult to determine if the active has any additional effect on top of this. In conclusion, it is unclear if any additional information could possibly be obtained in this photocarcinogenicity study if a larger aliquot of drug product had been applied in this study. Therefore, under the circumstances, it is my opinion that the conduct of this study is adequate.

Due to the significant enhancement of photocarcinogenesis observed with vehicle alone in this study, it is recommended that information be included in the label for this drug product as a safety measure for patients. It is recommended that the results of the photo co-carcinogenicity study be included in the label. In addition, it is recommended that a cautionary statement be included in the label indicating that patients under treatment should minimize or avoid exposure to natural or artificial sunlight.

It is important to note that the sponsor submitted a report titled "Expert commentary on the preclinical photocarcinogenesis study: SDZ ASM 981 cream: 52 week photocarcinogenesis study in hairless mice" (T-129/BS-195) in the NDA submission. This commentary and evaluation of the photocarcinogenicity study was by [redacted] This report summarized the findings of the dose range studies for the photocarcinogenicity study and the findings from the photocarcinogenicity study. In addition, a final conclusion from the results of this study were that the enhanced photocarcinogenesis due to the vehicle was possibly due to an effect on the optical properties of the skin. No additional photocarcinogenesis beyond that noted for the vehicle was noted in ASM 981 cream treated groups. Therefore, the final recommendation was to provide a cautionary statement in the label warning to minimize exposure to the sun during use of this product. The information contained in this commentary parallels what was provided in this review and the final conclusion is in agreement with the current agency thinking on this issue.

Carcinogenicity Study #2:

Oncogenicity study by oral gavage administration to CD-1 mice for their life span

Study Title: Oncogenicity study by oral gavage administration to CD-1 mice for their life span
Study Number: T-126/BS-32

Note: The oral mouse carcinogenicity study conducted with ASM 981 was reviewed under IND [redacted] and will be summarized in this review.

The study design for the mouse oral carcinogenicity study conducted in CD-1 mice is provided in the following table.

Study Design

Group	Treatment	Dose (mg/kg/day)	Number of Main Study Animals		Number of Toxicokinetic Animals	
			Males	Females	Males	Females
1	Vehicle Control	0	60	60	24	24
2	Vehicle Control	0	60	60	24	24
3	ASM 981	1	60	60	24	24
4	ASM 981	5	60	60	24	24
5	ASM 981	15	60	60	24	24
6	ASM 981	45	60	60	24	24

Test article was administered via oral gavage (10 ml/kg) on a daily basis. The vehicle solution was composed of: Hydroxypropylmethyl cellulose 3 [redacted] g/100 g vehicle), Poloxamer 188 [redacted] g/100 g vehicle) and Reverse Osmosis water [redacted] g/100 g vehicle).

Dose selection for the mouse oral carcinogenicity study was made based on the results of the 13 week oral toxicity study conducted in mice. Pancreas, preputial gland, vagina, uterus, spleen, mesenteric lymph nodes and thymus were identified as target organs in the 13 week oral toxicity study in mice. Minimal to slight vacuolation was noted in the pancreas of high dose females. This effect correlated with a slight increase in serum glucose in two animals in this group. Dose dependent minimal to massive atrophy of the preputial gland was present in males at doses ≥ 50 mg/kg/day. Alteration of the cycle related histomorphological changes in the vagina were noted in a few high dose females. The vaginal alterations were associated with slight uterus atrophy in some affected females. Hyperplastic changes in the thymic cortex and pleomorphic lymphoid cell proliferation in the spleen were noted at ≥50 mg/kg/day. Malignant lymphoma in the spleen, lymph nodes and thymus was observed at ≥100 mg/kg/day. Treatment related systemic immunosuppression, manifested as medullary atrophy in the thymus and reductions in lymphocytes in a few animals, was evident at ≥50 mg/kg/day. NOAEL = 10 mg/kg/day [$AUC_{(0,5-4\ hr)}$ = 1028 (M) and 2949 (F) ng·h/ml].

High levels of mortality were noted in mid-high dose females and high dose animals. The high dose group was terminated when the number of animals in the group reached 25. High dose females and males were sacrificed during week 87 and 91, respectively. The remaining females on the study were sacrificed after 91 weeks (when the number of surviving mid-high dose females reached 25). The remaining males on study were sacrificed after 104 weeks.

Toxicokinetic analysis demonstrated relatively high systemic exposure of mice to ASM 981 (refer to table below for more detail). The target organs of toxicity identified in this study (thymus, spleen and mandibular lymph node) mirror those that have been identified in other oral repeat dose toxicity studies conducted in mice with ASM 981.

The mean plasma pharmacokinetic parameters of ASM 981 after oral gavage administration on Weeks 4, 48 and 70 in CD-1 mice are summarized in the following table.

Dose (mg/kg)	Sex	C_{max} (ng/ml) Mean			T_{max} (hr) Mean			AUC_{0-24hr} (ng•hr/ml) Mean		
		Week 4	Week 48	Week 70	Week 4	Week 48	Week 70	Week 4	Week 48	Week 70
1	M	4	5	7	1.5	1.5	1.5	27	29	81
1	F	10	12	17	0.5	0.5	0.5	24	51	76
5	M	155	118	60	0.5	1.0	0.5	451	369	396
5	F	200	196	223	0.5	0.5	0.5	518	793	951
15	M	1042	1414	855	0.5	0.5	0.5	3580	4372	2260
15	F	638	1348	1326	0.5	0.5	0.5	2059	7662	5059
45	M	2297	2513	2053	0.5	0.5	0.5	13548	12650	9821
45	F	2475	2815	2204	0.5	0.5	0.5	10959	18450	12911

Peak blood concentrations were found between 0.5 and 1.5 hours post dosage. Mean peak concentrations (C_{max}) were between 4 and 2475 ng/ml for Week 4, between 5 and 2815 ng/ml for Week 48 and between 7 and 2204 ng/ml for Week 70. AUC_{0-24hr} values showed a dose dependent increase. There was a greater than proportional increase with the dose for both genders. This effect was more prominent after 4 weeks of treatment and became less prominent after weeks 48 and 70 of treatment between the 15 mg/kg/day and 45 mg/kg/day dose groups. In general, female satellite study mice appeared to have higher systemic exposure during the 70 week treatment period by consideration of AUC_{0-24hr} and C_{max} values.

Malignant lymphoma was noted as a treatment related neoplastic lesion in this study. The types of lymphoma were subdivided in the final study report on the basis of the constituent, predominant cell type and the extent of architectural distortion by the neoplastic lesion. The subtypes encountered in this study were lymphocytic/lymphoblastic lymphoma, immunoblastic lymphoma, follicular center cell lymphoma and pleomorphic lymphoma. The latter type was characterized by a mixture of different lymphoid cell types without any one type predominating. The final study report states that this broad classification of lymphomas is based on that described by Pattengale and Frith. The incidence of malignant lymphoma in this study is provided in the following table.

Incidence of Malignant Lymphoma

Note: Groups 1, 2, 3, 4, 5 and 6 represent Control 1, Control 2, 1 mg/kg/day ASM 981, 5 mg/kg/day ASM 981, 15 mg/kg day ASM 981 and 45 mg/kg/day ASM 981, respectively.

Lymphoma Subtype	Males						Females					
	1	2	3	4	5	6	1	2	3	4	5	6
Lymphocytic/ Lymphoblastic	1/60	5/60	0/60	3/60	1/60	2/60	8/60	4/60	5/60	6/60	4/60	4/60
Follicular center cell	1/60	3/60	3/60	3/60	6/60	7/60	10/60	6/60	4/60	8/60	13/60	17/60
Pleomorphic cell	0/60	0/60	0/60	0/60	1/60	8/60	1/60	0/60	0/60	0/60	1/60	6/60
Immunoblastic lymphoma	1/60	0/60	0/60	0/60	0/60	0/60	--	--	--	--	--	--
Total Malignant Lymphomas	**3/60**	**8/60**	**3/60**	**6/60**	**8/60**	**17/60**	**19/60**	**10/60**	**9/60**	**14/60**	**18/60**	**27/60**

The statistical analysis for this study included the high dose group since most of the animals (58.3%) in the high dose group survived for at least 78 weeks. The dose-response trend analysis of follicular center cell lymphoma ($p = 0.0009$; $p = 0.0004$ females) and pleomorphic lymphoma ($p = 0.0019$ males; $p = 0.0000$ females) in males and females were found to be statistically significant. The dose response trend analysis of the combined incidences of follicular center cell lymphoma, pleomorphic lymphoma, immunoblastic lymphoma and lymphocytic/ lymphoblastic lymphoma was found to be statistically significant in both sexes ($p<0.001$ in both sexes). Analysis after excluding the 45 mg/kg/day group did not show a statistically significant dose response trend. Pairwise comparisons showed a statistically significant increased incidence of follicular center cell lymphoma ($p = 0.007$ males, $p = 0.024$ females) and pleomorphic lymphoma ($p = 0.028$ males; $p = 0.003$ for females) and combined lymphoma ($p = 0.004$ males, $p = 0.005$ females) in the 45 mg/kg/day dose group males and females compared to control animals.

In summary, the total number of malignant lymphomas was statistically significantly increased in high dose animals (45 mg/kg/day) compared to control animals. In particular the number of pleomorphic lymphoma and follicular center cell lymphoma were statistically significantly increased in high dose animals compared to control animals. The incidence of follicular center cell lymphomas appeared to be increased for mid-high dose animals (15 mg/kg/day) compared to control animals. However, this did not reach statistical significance under the conditions of this study.

The dose response trend analysis of the combined incidences of follicular center cell lymphoma, pleomorphic lymphoma, immunoblastic lymphoma and lymphocytic/ lymphoblastic lymphoma was found to be statistically significant in both sexes ($p<0.001$ in both sexes). This is not a surprising finding based on the pharmacology of ASM 981, an immunosuppressant agent. Significant systemic absorption was noted in this study. The level of systemic ASM 981 exposure is probably high enough to cause systemic immunosuppression.

It has been established in the literature that an increased incidence of malignancy is a recognized complication of systemic immunosuppression therapy. The most common forms of neoplasm are non-Hodgkin's lymphomas and carcinomas of the skin.

An Exec CAC meeting was conducted on 6-19-01 to discuss the results from all of the carcinogenicity studies conducted for ASM 981. The minutes from the Exec CAC meeting are included in the addendum list provided later in this review. The committee concurred that there was a strong signal for malignant lymphoma in this study. The committee noted that hyperplastic changes were seen in the thymus at higher doses (≥ 50 mg/kg/day) in the 13 week repeat dose oral toxicity study in mice. The committee expressed concern that thymomas might have been seen at a true MTD dose in the mouse oral carcinogenicity study. The committee recommended that the malignant lymphoma findings noted in this study and the information concerning the short latency of malignant lymphoma formation in mice after repeat oral dosing be included in the label if the drug product is approved.

The highest measured $AUC_{(0\text{-}24\ hr)}$ value measured in humans that applied 1% ASM 981 cream was 38 ng·hr/ml. This was measured in a single pediatric patient that applied 1% ASM 981 cream bid to 43.5% BSA. The multiple of human exposure will be calculated based on this highest $AUC_{(0\text{-}24\ hr)}$ value. The NOAEL for lymphoma formation is the 15 mg/kg/day dose group ($AUC_{(0\text{-}24\ hr)}$ for males = 2260 ng·hr/ml after week 70 of treatment; $AUC_{(0\text{-}24\ hr)}$ for females = 5059 ng·hr/ml after week 70 of treatment). The multiple of human exposure ranges from 60 – 133X based on the NOAEL $AUC_{(0\text{-}24\ hr)}$ levels identified in this oral mouse carcinogenicity study. In my opinion, this provides an adequate safety margin for the potential concern of lymphoma formation in humans after use of 1% ASM 981 cream under maximum use conditions.

It is recommended that the tumor findings of this study and the multiples of human exposure levels be included in the label. In addition, it is recommended that the short latency of malignant lymphoma formation in mice after repeat oral dosing be included in the label (per the Exec CAC recommendation).

Carcinogenicity Studies #3 & 4:

Oncogenicity study by oral gavage administration to —— Wistar rats for 104 weeks

Oncogenicity study by oral gavage administration to —— Wistar rats for 104 weeks

Study #3 Title: Oncogenicity study by oral gavage administration to —— Wistar rats for 104 weeks

Study #3 Number: T-130/BS-381

Study #4 Title: Oncogenicity study by oral gavage administration to —— Wistar rats for 104 weeks

Study #4 Number: T-131/BS-358

Note: The oral rat carcinogenicity studies conducted with ASM 981 were reviewed under IND ——— and will be summarized in this review.

The study design for the rat oral carcinogenicity study conducted in Wistar rats is provided in the following table.

Study 1 Design

Group	Treatment	Dose (mg/kg/day)	Number of Main Study Animals		Number of Toxicokinetic Animals	
			Males	Females	Males	Females
1	Vehicle Control	0	60	60	14	14
2	Vehicle Control	0	60	60	14	14
3	ASM 981	1	60	60	14	14
4	ASM 981	5	60	60	14	14
5	ASM 981	25	60	60	14	14

Study 2 Design

Group	Treatment	Dose (mg/kg/day)	Number of Main Study Animals		Number of Toxicokinetic Animals	
			Males	Females	Males	Females
1	Vehicle Control	0	60	60	14	14
2	Vehicle Control	0	60	60	14	14
3	ASM 981	10	60	60	14	14

Test article was administered via oral gavage (5 ml/kg) on a daily basis. The vehicle solution was composed of: Hydroxypropylmethyl cellulose 3 — (— g/100 g vehicle), Poloxamer 188 (— g/100 g vehicle) and Reverse Osmosis water — g/100 g vehicle).

The dose selection for the first oral carcinogenic assay in rats was based on the results of the 26 week oral toxicity study conducted in rats (0, 1, 5, and 25 mg/kg/day ASM 981). One high dose male rat was sacrificed in extremis on day 107 due to the clinical signs of weak legs and emaciation and one high dose female on day 118 due to convulsions and jumping. Malignant reticulosis was observed in the brain and spinal cord in the male and moderate encephalitis in the female upon histopathological examination. Moderate impairment of body weight gain associated with slightly increased food consumption was noted in high dose males.

ASM 981 demonstrated effects on the lymphoreticular system, which was expected based on its pharmacological immunosuppressive action. Reduced lymphocyte counts and medullary atrophy in the thymus were indicative of suppressive activity on the immune system. Inflammatory cell infiltration and edema in the glandular stomach was noted in high dose animals. Functional and/or morphological changes were noted in the kidneys and pancreas of

high dose animals. The functional kidney changes were expressed as low urine specific gravity, loss of electrolytes, decreased creatinine clearance and increased serum urea and creatinine concentrations. Morphological changes in the kidney were characterized by an increased incidence of basophilic tubuli and an increased incidence and severity of corticomedullary mineralization. Morphological changes in the pancreas were noted as vacuolation and reduction in the number of islet cells. An increased incidence of lens cataracts was noted after chronic treatment in high dose animals.

ASM 981 showed effects on reproductive organs in high dose animals. These effects included reduced prostate gland weight, epithelial atrophy of seminal vesicles, suppression of estrus cycle, vaginal and uterine atrophy. The sponsor stated that these findings were indicative of altered sex hormone function in male and female rodents. The sponsor conducted an additional investigation oral 4 week study in rats to examine this effect. The results from this study suggest that ASM 981 treatment resulted in low levels of estrogen and testosterone. An early stage benign thymoma was diagnosed upon microscopic examination in the thymus of 1/40 rats in the high dose group.

The NOAEL was identified as 5 mg/kg/day in this 26 week oral toxicity study in rats.

The dose for the second carcinogenicity study was selected to provide an intermediate dose between the mid dose (5 mg/kg/day ASM 981) and the high dose (25 mg/kg/day ASM 981) used in the first oral rat carcinogenicity study. A high mortality rate was noted in high dose animals in the first oral rat carcinogenicity.

Mortality was high in high dose animals in the first study compared to control animals. The high dose group was terminated early (week 58) when the number of survivors had fallen to 25. The mortality of animals in the low and mid dose groups appeared to not be affected by treatment. In the second study, mortality was high in 10 mg/kg/day ASM 981 treated animals compared to control animals. Males were sacrificed after 88 weeks of treatment when the number of survivors in the ASM 981 treated group fell to 25. Females completed the scheduled 104 weeks of treatment.

Toxicokinetic analysis demonstrated relatively high systemic exposure of rats to ASM 981 (refer to table below for additional detail). The target organs of toxicity identified in the two oral rat carcinogenicity studies (eyes, pancreas, kidneys, mesenteric lymph node, thymus and uterus) mirror those that have been identified in other oral repeat dose toxicity studies conducted in rats with ASM 981.

The mean plasma pharmacokinetic parameters of ASM 981 in the first study after oral gavage administration on Weeks 4, 48 and 72 in Wistar rats are summarized in the following table.

Dose (mg/kg)	Sex	C_{max} (ng/ml) Mean			T_{max} (hr) Mean			AUC_{0-24hr} (ng•hr/ml) Mean		
		Week 4	Week 48	Week 72	Week 4	Week 48	Week 72	Week 4	Week 48	Week 72
1	M	3.2	6.4	22	1.0	1.0	0.5	7.8	14	42
1	F	2.1	4	29	0.5	1.0	0.5	2.8	5.1	21
5	M	72	263	417	1.0	1.0	0.5	234	981	1222
5	F	135	212	322	0.5	0.5	0.5	281	486	805
25	M	861	2070	--	2.0	2.0	--	3576	7846	--
25	F	1933	3030	--	2.0	1.0	--	14801	15262	--

Peak blood concentrations were found between 0.5 and 2.0 hours post dose. Mean peak concentrations (C_{max}) were between 2.1 and 1933 ng/ml for Week 4, between 4 and 3030 ng/ml for Week 48 and between 22 and 417 ng/ml for Week 72 (lower C_{max} values due to the high dose group deleted at this timepoint). AUC_{0-24hr} values showed a dose dependent increase and there appeared to be an increase in AUC over proportionally to dose. Overall, the AUC and C_{max} values tended to be higher after 72 weeks of treatment compared to after 4 weeks. For high dose animals, the female AUC and C_{max} values tended to be higher than the male values. However, for low and mid dose animals, this trend was reversed with male AUC and C_{max} values higher than female values.

The mean plasma pharmacokinetic parameters of ASM 981 in the second study after oral gavage administration in Wistar rats are summarized in the following table.

Dose (mg/kg)	Sex	C_{max} (ng/ml) Mean			T_{max} (hr) - Mean			AUC_{0-24hr} (ng•hr/ml) Mean		
		Week 4	Week 48	Week 88 or 104*	Week 4	Week 48	Week 88 or 104*	Week 4	Week 48	Week 88 or 104*
10	M	292	433	486	2	2	1	1422	1663	1521
10	F	517	611	257	0.5	2	0.5	1587	3187	1550

* - Plasma samples were obtained during week 88 for male animals and during week 104 for female animals.

Peak blood concentrations were found between 0.5 and 2.0 hours post dose. The mean peak concentrations (C_{max}) and AUC_{0-24hr} values were between those noted for the 5 and 25 mg/kg/day ASM 981 dose groups in the previous rat oral carcinogenicity study.

Benign thymoma in the thymus was noted as a treatment related neoplastic lesion in study 1. The incidence of benign thymoma from study 1 is provided in the following table.

Incidence of Neoplastic Lesions from Study 1

Note: Groups 1, 2, 3, 4 and 5 represent Control 1, Control 2, 1 mg/kg/day ASM 981, 5 mg/kg/day ASM 981 and 25 mg/kg/day ASM 981, respectively.

Neoplastic Lesion	Males					Females				
	1	2	3	4	5	1	2	3	4	5
Thymus										
Benign thymoma	4/60	3/60	4/60	9/60	1/59	8/58	7/60	4/59	6/60	6/60

The statistical analysis for this study excluded the high dose group due to the early sacrifice of the high dose group (<78 weeks). In addition, since both the control groups were handled identically and no significant mortality difference was detected between them, the two controls were combined for the statistical analysis of this study.

A higher incidence of benign thymoma in the thymus was noted in mid dose male animals compared to control males. However, no increase in any tumor type was considered statistically significant based on the dose response trend analysis (thymoma trend p values: p=0.026 for males; p = 0.680 for females and uterine polyp trend p value: p = 0.107) or the pairwise comparison analysis (thymoma pairwise p values for mid dose animals compared to control animals: p = 0.046 males; p = 0.838 females).

Even though the higher incidence in benign thymoma in mid dose males was not statistically significant, this does indicate that perhaps a more significant effect could be noted in a dose group between the mid and high dose groups tested in this study. The sponsor did test an intermediate dose (10 mg/kg/day) in a second oral rat carcinogenicity study, which is reviewed below.

Based on data submitted with this carcinogenicity study, the historical percentage range of benign thymoma in male wistar rats is 0.0% - 8.3% and for female wistar rats is 0.0 – 18.6% in the laboratory that conducted the study. The incidence of benign thymoma in mid dose male animals was 9/60 (15%) and in mid dose female animals was 6/60 (10%). The incidence of benign thymoma noted in high dose male rats is 1.8 fold greater than the high end of the incidence range noted in control rats (15% vs 8.3%). The incidence of benign thymoma noted in high dose female rats is 54% of the value for the high end of the incidence range noted in control female rats (10% vs 18.6%). Therefore, it could be interpreted that a potential trend for benign thymoma has been noted in mid dose male rats (even though this did not reach statistical significance) but not mid dose female rats.

Benign thymoma in the thymus was noted in the second study as a treatment related neoplastic lesion in this study. The incidence of benign thymoma in this study is provided in the following table.

Incidence of Neoplastic Lesions

Note: Groups 1, 2 and 3 represent Control 1, Control 2 and 10 mg/kg/day ASM 981, respectively.

Neoplastic Lesion	Males			Females		
	1	2	3	1	2	3
Thymus						
Benign thymoma	1/60	2/60	7/60	9/60	6/60	17/60

Since both the control groups were handled identically and no significant mortality difference was detected between them, the two controls were combined for the statistical analysis of this study.

A higher incidence of benign thymoma in the thymus was noted in ASM 981 treated animals compared to control animals. The increased incidence of thymoma was considered to be statistically significantly greater compared to control animals ($p = 0.0005$ for males; $p = 0.0001$ for females). The study report describes the benign thymoma as tumors of thymic epithelial cells with varying proportions of participating lymphocytes.

Based on the data provided under the previous oral rat carcinogenicity study, the historical percentage range of benign thymoma in male Wistar rats is 0.0% - 8.3% and for female Wistar rats is 0.0 – 18.6%. The incidence of benign thymoma in 10 mg/kg ASM 981 treated male animals was 7/60 (11.7%) and in 10 mg/kg ASM 981 treated female animals was 17/60 (28.3%). The incidence of benign thymoma noted in high dose male rats is 1.4 fold greater than the high end of the incidence range noted in control rats (11.7% vs 8.3%). The incidence of benign thymoma noted in high dose female rats is 1.5 fold greater than the value for the high end of the incidence range noted in control female rats (28.3% vs 18.6%). Therefore, a positive signal for benign thymoma was noted in 10 mg/kg ASM 981 treated male and female animals. In addition, the increased incidence of benign thymoma in high dose animals was statistically significantly greater than in control animals. It is important to note that the incidence of benign thymoma in 10 mg/kg/day male rats (11.7%) was not greater than that noted in 5 mg/kg/day male rats (15%). The reason for this is probably related to the duration of treatment. The 5 mg/kg/day dosed rats were treated for 104 weeks and the 10 mg/kg/day dosed rats were treated for 88 weeks. If the 10 mg/kg/day dosed rats had been treated longer (until there were 10 animals in this group), then the incidence rate for benign thymoma may have been greater than the 11.7% value.

It is important to note that the sponsor submitted a report titled "Toxicological evaluation of thymic tumors in a carcinogenicity test of SDZ ASM 981 in —— Wistar rats" (T-132/BS-739) in the NDA submission. The sponsor believes that the increased incidence of benign thymoma is a species and strain specific finding and is not considered to represent a risk to patients exposed to ASM 981 by the dermal or oral route. It has been established that Wistar rats do have a fairly high incidence of thymomas. In this report, the author ———— compares the incidence of thymoma in the oral rat

carcinogenicity study with the incidence rate established in the RITA database of findings in control animals collected from European laboratories. The RITA database reports an incidence range of benign thymoma in male (n = — and female (n = —— Wistar rats of —— and -·— respectively. The male incidence of benign thymomas in this study (5 mg/kg/day = 15%; 10 mg/kg/day = 11.7%) would have fallen within the RITA control incidence range for male Wistar rats. However, the 10 mg/kg/day dose female incidence of benign thymomas in this study (28.6%) was still greater than the RITA control incidence range for female Wistar rats. In general, it is more accurate to compare the treated incidence rate for a particular tumor type to the control incidence rate established in the conducting laboratory. Therefore, the comparison to the RITA database is not appropriate for this study. In this report, the author proposes that the increased incidence of benign thymoma is related to an alteration in the hormonal status of the rat. Another possibility could be related to the pharmacologic action of ASM 981 (an immunosuppressive agent) that has been show to target the thymus as a target organ of toxicity. The increased incidence of benign thymoma may be directly related to the immunosuppressive effects of ASM 981.

An Exec CAC meeting was conducted on 6-19-01 to discuss the results from all of the carcinogenicity studies conducted for ASM 981. The minutes from this meeting are included in the addendum list below. The committee determined that a MTD had been reached in this study and that this was an adequate study. The committee concurred that there was a signal for benign thymoma in this study. The committee commented that the incidence of benign thymoma in 5 mg/kg/day treated males might have reached statistical significance if the two studies had been combined prior to statistical analysis. Therefore, the committee stated that the NOAEL for benign thymoma in male rats is 1 mg/kg/day and in female rats is 5 mg/kg/day. The committee commented that the finding of benign thymoma in this study does not seem irrelevant in conjunction with the hyperplastic changes noted in the thymus in the 13 week repeat dose oral toxicity study in mice. The committee recommended that the benign thymoma findings noted in this study be included in the label if the drug product is approved.

In summary, the NOAEL for female rats is 5 mg/kg/day and the NOAEL for male rats is 1 mg/kg/day after combining the results from the two rat oral carcinogenicity studies. The NOAEL for male rats is 1 mg/kg/day because perhaps statistical significance may have been achieved if the results of both studies had been combined prior to statistical analysis instead of analyzing each study separately. In addition, the increased incidence of benign thymoma in 5 mg/kg/day males was greater than the historical control level for benign thymoma in male Wistar rats. The formation of benign thymoma may be related to the pharmacology of ASM 981, an immunosuppressant agent. Significant systemic absorption was noted in this study. The level of systemic ASM 981 exposure was probably high enough in this study to cause systemic immunosuppression.

The highest measured $AUC_{(0\text{-}24\ hr)}$ value measured in humans that applied 1% ASM 981 cream was 38 ng·hr/ml. This was measured in a single pediatric patient that applied 1% ASM 981 cream bid to 43.5% BSA. The multiple of human exposure will be calculated based on this highest $AUC_{(0\text{-}24\ hr)}$ value. The NOAEL for benign thymoma formation is 1 mg/kg/day in male rats ($AUC_{(0\text{-}24\ hr)}$ for males = 42 ng·hr/ml after week 72 of treatment) and 5 mg/kg/day in female rats ($AUC_{(0\text{-}24\ hr)}$ for females = 805 ng·hr/ml after week 72 of treatment). The multiple of human

exposure ranges from 1.1 – 21X based on the NOAEL $AUC_{(0\text{-}24\ hr)}$ levels identified in this oral rat carcinogenicity study. In my opinion, the multiple of human exposure based on the NOAEL for female mice does provide for an adequate safety margin for the potential concern of benign thymoma formation in humans after use of 1% ASM 981 cream under maximum use conditions. However, the multiple of human exposure based on the NOAEL for male mice does not provide for an adequate safety margin. However, it must be taken into consideration that the majority of the human pharmacokinetic measurements after repeat dose administration of the 1% ASM 981 cream were below the level of detection. Therefore, the actual potential risk of thymoma formation may be much less than the estimate calculated based on the highest measured $AUC_{(0\text{-}24\ hr)}$ value measured in humans that applied 1% ASM 981 cream.

It is recommended that the tumor findings of this study and the multiples of human exposure levels be included in the label.

Carcinogenicity Study #5:

Carcinogenicity study by dermal administration to mice

Study Title: Carcinogenicity study by dermal administration to mice
Study Number: T-127/BS-530

Note: The dermal mouse carcinogenicity study conducted with ASM 981 dissolved in ethanol was reviewed in more detail in an addendum review to the NDA and will be summarized in this review.

The study design for the mouse dermal carcinogenicity study conducted in CD-1 mice is provided in the following table.

Study Design

ASM 981 Concentration (%)		ASM 981 Dose (mg/kg/day)	Number of Main Study Animals		Number of Toxicokinetic Animals	
Week 1-3	Week 4-end		Males	Females	Males	Females
NA	NA	Untreated Control	50	50	24	24
0	0	Vehicle Control	50	50	24	24
0.0027	0.0032	0.04	50	50	24	24
0.027	0.032	0.40	50	50	24	24
0.27[a]	0.32[b]	4.00	50	50	24	24

Note: ASM 981 was dissolved in ethanol as the vehicle for this study.

a – high dose stock concentration was 2.7 mg/ml in ethanol and lower dose groups were derived from ———————

b – high dose stock concentration was 3.2 mg/ml in ethanol and lower dose groups were derived from [redacted]

A 2 x 3 cm area, estimated to be at least 10% of the total body surface, was clipped with an electric clipper. The clipped zone included the dorsal retro-scapular region, on both sides of the spinal column, the back and two flanks. The animals were clipped on an as needed basis throughout the study. The test article was administered by dermal application using an adjustable volume pipette fitted with a plastic tip. No massage was performed but the tip was used to spread the formulation over the application site. The animals were not rinsed between each application and no dressing was applied to the application site. Untreated animals received no treatment but were clipped as frequently as the other animals. The volume of application of the formulations was 1.5 ml/kg/day during the first three weeks and 1.25 ml/kg/day thereafter. The total application volumes were generally lower than 50 µl in this study.

Note: The highest percentage of ASM 981 in ethanol applied in this study was 0.32%. This is about 1/3 of the concentration that will be used clinically (1% ASM 981 cream). It would have been preferable to have the high dose set at 1% or higher, if possible.

The basis for dose selection in this study was not acceptable by agency standards. Dose selection was not based on MTD or Maximum Feasible Dose. The highest dose was selected as a dose that would not produce lymphoma based on the results of the 13 week dermal mouse study. No CAC concurrence was obtained for the doses selected in this study.

It would have been preferable to have conducted this mouse dermal carcinogenicity study with the final marketed formulation of ASM 981 instead of with ASM 981 dissolved in ethanol. However, this mouse dermal carcinogenicity study was in progress at the time of the IND submission. The sponsor was informed shortly after the IND was submitted that the dermal carcinogenicity study would need to be conducted with the final marketed formulation of ASM 981. The sponsor conducted a second dermal carcinogenicity study in rat with the ASM 981 cream (final marketed formulation). The details and results of the second dermal carcinogenicity study in rats are presented later in this document.

The only toxic effects noted in this study was a slight decrease of food consumption at all dose levels and a slight reduction in body weight in mid and high dose males. No treatment related neoplastic or non-neoplastic findings were noted in this study. The conclusion reached by the statistical reviewer was the following. No statistically significant dose response or increased incidence of any tumor type in the high dose group was found when compared with either control based on results of Lin and Rahman for dose-response (trend analysis) and that of Haseman for pairwise comparisons. Therefore, the NOAEL for carcinogenicity effects is 4.0 mg/kg/day under the conditions of this study. The average AUC for the NOAEL was 1080 ng·hr/ml after 52 weeks of treatment.

It is interesting to note that the AUC levels obtained in the high dose group in this dermal mouse carcinogenicity study were significantly less than the NOAEL dose for lymphoma identified in the oral mouse carcinogenicity study. The NOAEL AUC values in the oral mouse carcinogenicity study for males and females were 2204 and 5059 ng·hr/ml, respectively, after 70

weeks of treatment. The NOAEL AUC values in the oral mouse carcinogenicity were about 2X – 5X greater than the AUC obtained in the high dose group in the dermal mouse carcinogenicity study. Therefore, it is not surprising that no lymphoma was noted in this dermal mouse carcinogenicity study.

An Exec CAC meeting was conducted on 6-19-01 to discuss the results from all of the carcinogenicity studies conducted for ASM 981. The minutes from the Exec CAC meeting are included in the addendum list provided below. The committee felt that this study was negative. However, the committee commented that the test article had obvious effects after dermal administration in mice (malignant lymphoma noted in the 13 week repeat dermal toxicity study in mice at doses ≥ 50 mg/kg/day). The committee recommended that this information be included in the label if the drug product is approved. In addition, the committee asked about the source of the metastatic carcinoma noted in the thymus of one high dose male. A request was sent to the sponsor on 7-10-01 to clarify the source of this metastatic tumor.

The highest measured $AUC_{(0\text{-}24\ hr)}$ value measured in humans that applied 1% ASM 981 cream was 38 ng·hr/ml. This was measured in a single pediatric patient that applied 1% ASM 981 cream bid to 43.5% BSA. The multiple of human exposure will be calculated based on this highest $AUC_{(0\text{-}24\ hr)}$ value. The NOAEL identified in this study is 4 mg/kg/day (average $AUC_{(0\text{-}24\ hr)}$ = 1080 ng·hr/ml after 52 weeks of treatment). The multiple of human exposure is 28X based on the NOAEL $AUC_{(0\text{-}24\ hr)}$ levels identified in this dermal mouse carcinogenicity study.

This multiple of human exposure probably provides an adequate safety margin for the potential concern of lymphoma formation in humans after use of 1% ASM 981 cream under maximum use conditions. Even though the high dose group was not selected according to current agency standards, it did provide for a systemic exposure level that was 28X the highest level obtained in humans under maximum use conditions. Therefore, it is unclear how much additional information would be obtained by requesting that the sponsor repeat the mouse dermal carcinogenicity study using higher doses that would elicit a positive signal for lymphoma. The NOAEL level for lymphoma formation has been established in the mouse from the oral mouse carcinogenicity study. In addition, lymphoma was noted in the 13 week dermal toxicity study in mice at the 50 mg/kg/day dose level. Therefore, it is recommended that the findings from the 13 week dermal toxicity study be included in the label to address the concern about lymphoma formation after dermal administration.

Carcinogenicity Study #6:

104-week dermal carcinogenicity study in rats

Study Title: 104-week dermal carcinogenicity study in rats
Study Number: T-133/BS-733

Note: The dermal rat carcinogenicity study conducted with 1% ASM 981 cream was reviewed in more detail in an addendum review to the NDA and will be summarized in this review.

The study design for the mouse dermal carcinogenicity study conducted in Wistar rats is

provided in the following table.

Study Design

ASM 981 Dose (%)	ASM 981 Dose (mg/kg/day)	Number of Main Study Animals		Number of Toxicokinetic Animals	
		Males	Females	Males	Females
Saline Control	0	50	50	10	10
Vehicle Control	0	50	50	10	10
0.2	2	50	50	10	10
0.6	6	50	50	10	10
1.0	10	50	50	10	10

Note: The ASM 981 cream used in this study is the same as the to be marketed formulation.

The back of each rat was shaved ~24 hours prior to the first dosing and then on a weekly basis during the course of the study. An intact skin area of ~20 cm^2 was selected from the shaved area for the administration site. Special jackets (supplier – ——, were used for each rat to fix the cover of the application site. Test article (1 gm/kg/day) was applied to the application site and spread as uniformly as possible. The application sites were covered with an insert, which was fixed to the jacket. Test article was gently washed off with lukewarm tap water after each daily 6 hour exposure period. Animals were treated with test article daily, 6 hours/day, 7 days/week for a duration of 104 weeks.

Note: It would have been preferable if the animals had been treated with test article for a 24 hour period instead of the 6 hour period. In addition, it would have been preferable if an untreated control group was used in this study instead of the saline control group.

The basis for dose selection in this study was Maximum Feasible Dose. The maximum feasible concentration of ASM 981 possible in the final marketed formulation is 1%. The sponsor stated that the amount applied of the test article (1 gm/kg/day) is the maximum volume that can be applied to the rat. Therefore, the high dose in this study was the maximum feasible dose that could be applied for ASM 981 in the final to be marketed cream formulation. No CAC concurrence was obtained for the doses selected in this study.

The only treatment related non-neoplastic finding noted in this study was an increased incidence of minimal to moderate epithelial hyperplasia at the application site. The incidence rate was similar in vehicle and ASM 981 treated groups. This effect was contributed to vehicle rather than ASM 981.

The agency's statistical reviewer evaluated the data by pairwise comparisons of the high dose group with the two control groups, per the pharm/tox reviewer request. The rationale for this request was because there was an incomplete analysis of all the tissues in the low and mid dose groups. Therefore, it would not be accurate to perform a trend test or pairwise comparisons of the low and mid dose groups under these conditions. Adjustments for pairwise comparisons

was done using the results of Haseman (1983) (i.e., use p=0.05 for rare tumors and p=0.01 for common tumor type).

The conclusion reached by the statistical reviewer was the following. A statistically significant increase in the incidence of follicular cell adenoma in the thyroid gland in the high dose group was found when compared with vehicle control (p = 0.0357) based on results of Haseman for pairwise comparisons. It is important to note that the follicular cell adenoma in the thyroid gland was considered a rare tumor for this statistical decision based on the incidence rate in the concurrent control group (i.e., the vehicle control group had an incidence of 0/50 and the high dose group had an incidence of 5/49). However, historical control data submitted by the sponsor per a request demonstrate that follicular cell adenoma of the thyroid gland is not a rare tumor in Wistar rats. Therefore, the increased incidence of follicular cell adenoma noted in high dose animals is not statistically significant according to adjustments based on Haseman for pairwise comparisons for a common tumor (i.e., the p value is > 0.01 for common tumors). A more detailed discussion of the historical incidence rates for follicular cell adenoma in the thyroid gland can be found in the addendum review for this NDA.

An Exec CAC meeting was conducted on 6-19-01 to discuss the results from all of the carcinogenicity studies conducted for ASM 981. The minutes from this meeting are included in the addendum list below. Incomplete histopathological analysis was performed in the low and mid dose groups in this study. The committee determined that a histopathological reanalysis of the thymus and thyroid from all low and mid dose animals is necessary to determine if the potential signal noted in these two dose groups is of potential concern or not. In addition, the committee requested a statistical reanalysis for the combined incidence for the follicular cell adenoma and follicular cell carcinoma of the thyroid. The committee inquired about the historical background incidence rate for follicular cell carcinoma of the thyroid for the strain of rat used in this study. A request was sent to the sponsor on 7-10-01 for the additional information needs. A statistical reanalysis will be performed after the requested information is submitted to the agency.

No rare or uncommon tumors were noted in this study. In addition, no statistically significant increase in any common tumors was detected in this study. All of the potential common neoplastic microscopic findings were within the historical control background incidence rate ranges for Wistar rats from the conducting laboratory. Therefore, it can be concluded that no significant treatment related effects on neoplastic microscopic findings were noted in this study. No significant toxicity was noted in this rat dermal carcinogenicity study. Therefore, the NOAEL can be set at 10 mg/kg/day (average $AUC_{(0\text{-}24\ hr)}$ = 125 ng·hr/ml after 104 weeks of treatment).

The highest measured $AUC_{(0\text{-}24\ hr)}$ value measured in humans that applied 1% ASM 981 cream was 38 ng·hr/ml. This was measured in a single pediatric patient that applied 1% ASM 981 cream bid to 43.5% BSA. The multiple of human exposure will be calculated based on this highest $AUC_{(0\text{-}24\ hr)}$ value. The NOAEL identified in this study is 10 mg/kg/day (average $AUC_{(0\text{-}24\ hr)}$ = 125 ng·hr/ml after 104 weeks of treatment). The multiple of human exposure is 3.2X based on the NOAEL $AUC_{(0\text{-}24\ hr)}$ levels identified in this dermal rat carcinogenicity study.

Carcinogenicity summary:

A dramatic vehicle effect was observed on the median tumor onset (decreased time to tumor onset) in the photocarcinogenicity study. The vehicle induced enhancement tended to be greater in male mice as compared to female mice. No additional effect of ASM 981 cream treatment on tumor development beyond the vehicle effect was noted in this study. For female animals, there was actually a protective effect observed at all three dose levels of ASM 981 cream. The reason for this unclear. The vehicle enhancement of photocarcinogenesis has been noted in other photocarcinogenicity studies conducted in the literature and submitted to the agency. One potential explanation for this could be the modification of the optical quality of the skin with resulting enhancement of UVR penetration, which could lead to an increase in UVR induced skin tumors.

Malignant lymphoma was noted as a treatment related neoplastic lesion in the high dose group in the oral mouse carcinogenicity study. The dose response trend analysis of the combined incidences of follicular center cell lymphoma, pleomorphic lymphoma, immunoblastic lymphoma and lymphocytic/ lymphoblastic lymphoma was found to be statistically significant in both sexes ($p<0.001$ in both sexes). This is not a surprising finding based on the pharmacology of ASM 981, an immunosuppressant agent. Significant systemic absorption was noted in this study. The level of systemic ASM 981 exposure was probably high enough to cause systemic immunosuppression.

It has been established in the literature that an increased incidence of malignancy is a recognized complication of systemic immunosuppression therapy. The most common forms of neoplasm are non-Hodgkin's lymphomas and carcinomas of the skin.

The highest measured $AUC_{(0\text{-}24\ hr)}$ value measured in humans that applied 1% ASM 981 cream was 38 ng·hr/ml. This was measured in a single pediatric patient that applied 1% ASM 981 cream bid to 43.5% BSA. The multiple of human exposure will be calculated based on this highest $AUC_{(0\text{-}24\ hr)}$ value. The NOAEL for lymphoma formation is the 15 mg/kg/day dose group ($AUC_{(0\text{-}24\ hr)}$ for males = 2260 ng·hr/ml after week 70 of treatment; $AUC_{(0\text{-}24\ hr)}$ for females = 5059 ng·hr/ml after week 70 of treatment). The multiple of human exposure ranges from 60 – 133X based on the NOAEL $AUC_{(0\text{-}24\ hr)}$ levels identified in this oral mouse carcinogenicity study. In my opinion, this provides an adequate safety margin for the potential concern of lymphoma formation in humans after use of 1% ASM 981 cream under maximum use conditions.

A statistically significant increase in the incidence of benign thymoma was noted in 10 mg/kg ASM 981 treated animals compared to control animals in the oral rat carcinogenicity study. The increase in benign thymoma noted in ASM 981 treated male and female animals was greater than the high end of the historical control benign thymoma incidence rate for male and female animals. In addition, a biologically significant increase in thymoma (above the historical control level) was noted in 5 mg/kg/day ASM 981 treated male rats. It is important to note that the thymoma incidence in 5 mg/kg/day ASM 981 treated male rats may have reached statistical significance if the two studies had been analyzed statistically together rather than as two separate studies. The sponsor proposes that the formation of benign thymoma is related to the suppression of estrogen and testosterone in female and male rats, respectively. Another

possibility is that the formation of benign thymoma may be related to the pharmacology of ASM 981, an immunosuppressant agent. Significant systemic absorption was noted in this study. The level of systemic ASM 981 exposure was probably high enough to cause systemic immunosuppression. In addition, the Exec CAC members commented that the finding of benign thymoma in this study does not seem irrelevant in conjunction with the hyperplastic changes noted in the thymus in the 13 week repeat dose oral toxicity study in mice.

Combining the results from the two rat oral carcinogenicity studies, the NOAEL for female rats is 5 mg/kg/day and the NOAEL for male rats is 1 mg/kg/day. The NOAEL for male rats is 1 mg/kg/day because perhaps statistical significance may have been achieved if the results of both studies had been combined prior to statistical analysis instead of analyzing each study separately. In addition, the increased incidence of benign thymoma in 5 mg/kg/day males was greater than the historical control level for benign thymoma in male Wistar rats.

The highest measured AUC $_{(0\text{-}24\ hr)}$ value measured in humans that applied 1% ASM 981 cream was 38 ng·hr/ml. This was measured in a single pediatric patient that applied 1% ASM 981 cream bid to 43.5% BSA. The multiple of human exposure will be calculated based on this highest $AUC_{(0\text{-}24\ hr)}$ value. The NOAEL for benign thymoma formation is 1 mg/kg/day in male mice ($AUC_{(0\text{-}24\ hr)}$ for males = 42 ng·hr/ml after week 72 of treatment) and 5 mg/kg/day in female mice ($AUC_{(0\text{-}24\ hr)}$ for females = 805 ng·hr/ml after week 72 of treatment). The multiple of human exposure ranges from 1.1 – 21X based on the NOAEL $AUC_{(0\text{-}24\ hr)}$ levels identified in this oral rat carcinogenicity study. In my opinion, the multiple of human exposure based on the NOAEL for female mice does provide for an adequate safety margin for the potential concern of benign thymoma formation in humans after use of 1% ASM 981 cream under maximum use conditions. However, the multiple of human exposure based on the NOAEL for male mice does not provide for an adequate safety margin. However, it must be taken into consideration that the majority of the human pharmacokinetic measurements after repeat dose administration of the 1% ASM 981 cream were below the level of detection. Therefore, the actual potential risk of thymoma formation may be much less than the estimate calculated based on the highest measured $AUC_{(0\text{-}24\ hr)}$ value measured in humans that applied 1% ASM 981 cream.

No signal for dermal or systemic carcinogenicity was noted in the dermal mouse carcinogenicity study. However, the doses selected for this study were not adequate according to agency standards. In addition, the vehicle for this study was ethanol instead of the to be marketed topical formulation vehicle. This also makes the design of this dermal mouse carcinogenicity study not adequate according to agency standards. The sponsor selected a high dose group (4 mg/kg/day) in the dermal mouse carcinogenicity study that would not cause lymphoma formation. The results of a study to investigate the dosage response of immunosuppression and lymphoproliferative disorders following dermal administration to CD-1 mice for 13 weeks was able to determine a NOAEL for lymphoproliferative changes. The NOAEL for lymphoproliferative changes was identified in this study as 10 mg/kg/day ($AUC_{0\text{-}24hr}$ = 643 and 675 ng·hg/ml for males and females, respectively) for mice after 13 weeks of topical administration of ASM 981 dissolved in ethanol. The lowest dose that a low incidence of lymphoproliferative changes was identified in this study was 25 mg/kg/day ($AUC_{0\text{-}24hr}$ = 1845 and 1745 ng·hg/ml for males and females, respectively) for mice after 13 weeks of topical administration of ASM 981 dissolved in ethanol.

It is interesting to note that the AUC levels obtained in the high dose group in this dermal mouse carcinogenicity study were significantly less than the NOAEL dose for lymphoma identified in the oral mouse carcinogenicity study. The NOAEL AUC values in the oral mouse carcinogenicity study for males and females were 2204 and 5059 ng·hr/ml, respectively, after 70 weeks of treatment. The NOAEL AUC values in the oral mouse carcinogenicity were about 2X – 5X greater than the AUC obtained in the high dose group in the dermal mouse carcinogenicity study. Therefore, it is not surprising that no lymphoma was noted in this dermal mouse carcinogenicity study.

The highest measured $AUC_{(0\text{-}24\ hr)}$ value measured in humans that applied 1% ASM 981 cream was 38 ng·hr/ml. This was measured in a single pediatric patient that applied 1% ASM 981 cream bid to 43.5% BSA. The multiple of human exposure will be calculated based on this highest $AUC_{(0\text{-}24\ hr)}$ value. The NOAEL identified in this study is 4 mg/kg/day (average $AUC_{(0\text{-}24\ hr)}$ = 1080 ng·hr/ml after 52 weeks of treatment). The multiple of human exposure is 28X based on the NOAEL $AUC_{(0\text{-}24\ hr)}$ levels identified in this dermal mouse carcinogenicity study. This multiple of human exposure probably provides an adequate safety margin for the potential concern of lymphoma formation in humans after use of 1% ASM 981 cream under maximum use conditions.

It is unclear how much additional information would be obtained by requesting that the sponsor repeat the mouse dermal carcinogenicity study using higher doses that would elicit a positive signal for lymphoma. The NOAEL level for lymphoma formation has been established in the mouse from the oral mouse carcinogenicity study. In addition, lymphoma was noted in the 13 week dermal toxicity study in mice at the 50 mg/kg/day dose level. Therefore, it is recommended that the findings from the 13 week dermal toxicity study be included in the label to address the concern about lymphoma formation after dermal administration.

No signal for dermal or systemic carcinogenicity was noted in the dermal rat carcinogenicity study. The rat dermal carcinogenicity study was conducted with the final to be marketed 1% ASM 981 cream. No rare or uncommon tumors were noted in this study. In addition, no statistically significant increase in any common tumors was detected in this study. All of the potential common neoplastic microscopic findings were within the historical control background incidence rate ranges for Wistar rats from the conducting laboratory. Therefore, it can be concluded that no significant treatment related effects on neoplastic microscopic findings were noted in this study. No significant toxicity was noted in this rat dermal carcinogenicity study. Therefore, the NOAEL can be set at 10 mg/kg/day (average $AUC_{(0\text{-}24\ hr)}$ = 125 ng·hr/ml after 104 weeks of treatment).

The highest measured $AUC_{(0\text{-}24\ hr)}$ value measured in humans that applied 1% ASM 981 cream was 38 ng·hr/ml. This was measured in a single pediatric patient that applied 1% ASM 981 cream bid to 43.5% BSA. The multiple of human exposure will be calculated based on this highest $AUC_{(0\text{-}24\ hr)}$ value. The NOAEL identified in this study is 10 mg/kg/day (average $AUC_{(0\text{-}24\ hr)}$ = 125 ng·hr/ml after 104 weeks of treatment). The multiple of human exposure is 3.2X based on the NOAEL $AUC_{(0\text{-}24\ hr)}$ levels identified in this dermal rat carcinogenicity study.

Carcinogenicity conclusions:

A photocarcinogenic effect (decreased time to tumor formation) was noted for vehicle treatment in the photo co-carcinogenicity study in mice.

The carcinogenicity studies (oral studies in mice and rats; dermal studies in mice and rats) conducted to support ASM 981 were adequate. 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. The multiple of human exposure for the oral mouse carcinogenicity study (ranged from 60 – 133X) based on the NOAEL $AUC_{(0\text{-}24\ hr)}$ levels provides an adequate safety margin for the potential formation of lymphoma. The multiple of human exposure for the oral rat carcinogenicity study ranged from 1.1 – 21X based on the NOAEL $AUC_{(0\text{-}24\ hr)}$ levels. In my opinion, the multiple of human exposure based on the NOAEL for female rats does provide for an adequate safety margin for the potential concern of benign thymoma formation in humans after use of 1% ASM 981 cream under maximum use conditions. However, the multiple of human exposure based on the NOAEL for male rats does not provide for an adequate safety margin. However, it must be taken into consideration that the majority of the human pharmacokinetic measurements after repeat dose administration of the 1% ASM 981 cream were below the level of detection. Therefore, the actual potential risk of thymoma formation may be much less than the estimate calculated based on the highest measured $AUC_{(0\text{-}24\ hr)}$ value measured in humans that applied 1% ASM 981 cream.

No signals for either dermal or systemic carcinogenicity were noted in the dermal mouse and rat carcinogenicity studies. 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 ASM 981 if the dose is high enough to allow sufficient systemic exposure to cause immunosuppression. It is important to note that the dermal mouse carcinogenicity study was conducted with ASM 981 dissolved in ethanol instead of the to be marketed cream formulation. Even though the dermal mouse carcinogenicity study was not conducted with an adequate dose range selection, the NOAEL dose in this study (4 mg/kg/day) did provide for a multiple of human exposure equal to 28X based on the NOAEL $AUC_{(0\text{-}24\ hr)}$ levels identified in this dermal mouse carcinogenicity study. This multiple of human exposure probably provides an adequate safety margin for the potential concern of lymphoma formation in humans after use of 1% ASM 981 cream under maximum use conditions.

The dermal rat carcinogenicity study was conducted with the final to be marketed ASM 981 cream product. The multiple of human exposure (3.2X) in the rat dermal carcinogenicity based on the NOAEL $AUC_{(0\text{-}24\ hr)}$ level did not provide as large a safety margin. However, the design of this study was adequate since the maximum feasible dose of the to be marketed cream formulation of ASM 981 was used as the high dose in this study. Therefore, this was the highest dose that could be tested in the rat dermal carcinogenicity study and even though the multiple of human exposure is not as great as noted in the other carcinogenicity studies, it is adequate under the conditions of this study.

Labeling Recommendations:

Due to the significant enhancement of photocarcinogenesis observed with vehicle alone in the photocarcinogenicity study, it is recommended that this information be included in the label for this drug product as a safety measure for patients. It is recommended that the results of the photo co-carcinogenicity study be included in the label. In addition, it is recommended that a cautionary statement be included in the label indicating that patients under treatment should minimize or avoid exposure to natural or artificial sunlight.

It is recommended that the tumor findings of the oral mouse (lymphoma) and rat (thymoma) carcinogenicity studies and the multiples of human exposure levels be included in the label. It is recommended that the short latency to lymphoma formation in mice after high dose oral exposure be included in the label. It is recommended that the findings from the 13 week dermal toxicity study in mice and corresponding multiples of human exposure levels be included in the label to address the concern about lymphoma formation after dermal administration.

Addendum/appendix listing:

- *CAC Report:*

An Exec CAC meeting to discuss all of the carcinogenicity studies conducted for ASM 981 (oral mouse, oral rat, dermal mouse and dermal rat) was held on 6-19-01. The minutes from that meeting are provided below.

Executive CAC
June 19, 2001

Committee: Joseph DeGeorge, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Jasti Choudary, B.V.Sc,. Ph.D., HFD-180, Alternate Member
Abby Jacobs, Ph.D., HFD-540, Supervisor
Barbara Hill, Ph.D., HFD-540, Presenting Reviewer

Author of Draft: Barbara Hill

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA # 21-302
Drug Name: Elidel (pimecrolimus) cream; 1% ASM 981 cream
Sponsor: Novartis Pharmaceuticals Corporation

Background:

ASM 981 is an anti-inflammatory/immunosuppressive ascomycin macrolactam derivative that is being developed for the topical treatment of moderate ——— Atopic Dermatitis. Atopic Dermatitis is primarily a pediatric indication and the duration of treatment is chronic. ASM 981 has undergone testing in a full battery of genotoxicity tests and showed no genotoxic potential. The sponsor has conducted an oral mouse carcinogenicity study, two oral rat carcinogenicity studies, a dermal mouse carcinogenicity study and a dermal rat carcinogenicity study. Exec CAC concurrence for the dose range selected in each of these studies was not obtained prior to the conduct of each study.

Mouse Oral Carcinogenicity Study:

A statistically significant increase in malignant lymphoma was noted in high dose male and female mice in the mouse oral (gavage) carcinogenicity study (refer to following table).

Incidence of Malignant Lymphoma in mice treated with ASM 981

Dose (mg/kg/day)	Males	Females
Vehicle Control 1	3/60	19/60
Vehicle Control 2	8/60	10/60
1	3/60	9/60
5	6/60	14/60
15	8/60	18/60
45	17/60	27/60

Rat Oral Carcinogenicity Studies:

Two rat oral (gavage) carcinogenicity studies were conducted for ASM 981. The doses used in the first study were 0 (Vehicle Control 1), 0 (Vehicle Control 2), 1, 5 and 25 mg/kg/day ASM 981. The doses used in the second study were 0 (Vehicle Control 1), 0 (Vehicle Control 2) and 10 mg/kg/day ASM 981. High mortality due to overt toxicity (males and females terminated after 58 weeks) was noted in high dose male and female rats in the first study. Therefore, the sponsor decided to conduct the second rat oral carcinogenicity study with a dose between the 5 and 25 mg/kg/day dose levels.

A statistically significant increase in benign thymoma was noted in 10 mg/kg/day treated male and female rats in the second rat oral carcinogenicity study. An increase in benign thymoma was noted in 5 mg/kg/day treated male rats in the first rat oral carcinogenicity study but did not reach statistical significance (the two rat oral carcinogenicity studies were not combined for statistical analysis). Therefore, the NOAEL for benign thymoma in male rats is 1 mg/kg/day and in female rats is 5 mg/kg/day. The incidence of benign thymoma for both rat oral carcinogenicity studies combined is provided in the following table.

Incidence of Benign Thymoma in rats treated with ASM 981

Study #	Dose (mg/kg/day)	Males	Females
1	Vehicle Control 1	4/60	5/58
1	Vehicle Control 2	3/60	7/60
2	Vehicle Control 1	1/60	9/60
2	Vehicle Control 2	2/60	6/60
1	1	4/60	4/59
1	5	9/60[a]	6/60
2	10	7/60[b]	17/60
1	25	1/59[c]	6/60

a – treated for 104 weeks; b – treated for 88 weeks; c – treated for 58 weeks

Mouse Dermal Carcinogenicity Study:

Doses tested in this study were 0 (untreated control), 0 (vehicle control), 0.04 (0.0032%), 0.40 (0.032%) and 4.0 (0.32%) mg/kg/day. ASM 981 was dissolved in ethanol for this mouse dermal carcinogenicity study. The highest concentration tested was 0.32% ASM 981 which is ~1/3 the concentration that will be used clinically (1% ASM 981 cream). It would have been preferable to have the highest dose be at least the 1% concentration and in the final to be marketed formulation. No signal for potential systemic or dermal carcinogenicity was noted in this study. However, the dose selection for this study was not adequate. The results of a 13 week dermal repeat dose toxicity study (ASM 981 dissolved in ethanol) demonstrated malignant lymphoma in the 50 mg/kg/day dose group. Therefore, the high dose selected for the mouse dermal carcinogenicity study was too low to have detected a possible malignant lymphoma signal.

Rat Dermal Carcinogenicity Study:

Doses tested in this study were 0 (saline control), 0 (vehicle control), 2 (0.2%), 6 (0.6%) and 10 (1.0%) mg/kg/day. The final to be marketed ASM 981 cream formulation was used in this rat dermal carcinogenicity study. The maximum feasible concentration of ASM 981 in the cream formulation (1%) was used as the high dose in this study. No apparent signal for potential systemic or dermal carcinogenicity was noted in this study. However, it is important to note that incomplete histopathological evaluation was performed for the low and mid dose groups in this study.

Executive CAC Recommendations and Conclusions:

Oral Mouse Carcinogencity Study:

1. The committee determined that a MTD was not achieved in this study.
2. The committee concurred that there was a strong signal for malignant lymphoma in this study.
3. The committee noted that hyperplastic changes were seen in the thymus at higher doses (≥ 50 mg/kg/day) in the 13 week repeat dose oral toxicity study in mice. The committee expressed concern that thymomas might have been seen at a true MTD dose in the mouse oral carcinogenicity study.
4. The committee recommended that the malignant lymphoma findings noted in this study be included in the label if the drug product is approved.
5. The committee recommended that information concerning the short latency of malignant lymphoma formation in mice after repeat oral dosing be included in the label if the drug product is approved.

Oral Rat Carcinogenicity Studies:

1. The committee determined that a MTD had been reached in this study.
2. The committee felt that this was an adequate study.
3. The committee concurred that there was a signal for benign thymoma in this study.
4. The committee commented that the finding of benign thymoma in this study does not seem irrelevant in conjunction with the hyperplastic changes noted in the thymus in the 13 week repeat dose oral toxicity study in mice.
5. The committee recommended that the benign thymoma findings noted in this study be included in the label if the drug product is approved.

Dermal Mouse Carcinogenicity Study:

1. The committee felt that this study was negative. However, the committee commented that the test article had obvious effects after dermal administration in mice (malignant lymphoma noted in the 13 week repeat dermal toxicity study in mice at doses ≥ 50 mg/kg/day). The committee recommended that this information be included in the label if the drug product is approved.
2. The committee asked about the source of the metastatic carcinoma noted in the thymus of one high dose male. A request will be sent to the sponsor to clarify the source of this metastatic tumor.

Dermal Rat Carcinogenicity Study:

1. The committee commented that the histopathological analysis for this study was incomplete since all the animals in the low and mid dose groups were not examined in this study. The committee did note that there may be a possible signal in the thyroid and/or thymus based on the incomplete histopathological data available for the low and mid dose groups.
2. The committee requested that the sponsor reanalyze the histopathology of the thyroid and thymus in all low and mid dose animals. In addition, the committee requested that another statistical analysis be performed after the data for the complete histopathological analysis of the thyroid and thymus in all low and mid dose animals has been submitted to the agency. A request for this histopathological re-analysis will be sent to the sponsor.
3. The committee recommended that a statistical reanalysis be performed for the combined incidence for the follicular cell adenoma and follicular cell carcinoma of the thyroid. The request for statistical reanalysis will be submitted to the biostatistical division in the agency after the complete histopathological reanalysis data has been submitted to the agency.
4. The committee requested the historical background incidence rate for follicular cell carcinoma of the thyroid for the strain of rat used in the dermal rat carcinogenicity study. This request will be sent to the sponsor.

General Comments:

1. [ ]

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

cc:

/Division File, HFD 540
/AJacobs/Sup, HFD-540
/BHill/Pharm, HFD-540
/MWright/PM, HFD-540
/ASeifried, HFD-024

APPEARS THIS WAY
ON ORIGINAL

REPRODUCTIVE TOXICOLOGY:

Reproductive Toxicology Study #1:

An oral reproductive toxicity dose range finding study in female rats with toxicokinetics and placental transfer (Drug form: lyophilisate suspension)

Study title: An oral reproductive toxicity dose range finding study in female rats with toxicokinetics and placental transfer (Drug form: lyophilisate suspension)

Key study findings: The results from this study suggested that the doses of 0, 2, 10 and 45 mg/kg/day ASM 981 would be adequate for the definitive oral fertility and embryo-fetal development studies in rats.

Study No.:	T-111/203-091
Sandoz Study No.:	1048R
Volume #, and page #:	68, 5-1
Conducting laboratory:	Sandoz Pharma Ltd., Basle, Switzerland
Date of study initiation:	11/4/94
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Drug, and lot#:	ASM 981 – batch# Y213 0794, Y289 1094 and Y272 0994
Formulation/vehicle:	Water & Plasmagelan^{R} (Note: ASM 981 lyophilisate was reconstituted using water and then further diluted with the plasma volume surrogate Plasmagelan^{R} to adjust to the final drug concentrations)

Methods:

Species/strain:	Female Wistar rats; 10 weeks old; 184 – 229 grams
Doses employed:	0, 12, 30, 45, 60 and 120 mg/kg/day (Note: A high percentage of resorptions was noted in the 60 and 120 mg/kg/day dose. Therefore, the 30 and 45 mg/kg/day dose groups were added to the study.
Route of administration:	Oral (gavage); Dose volumes = 12, 1.2, 3.0, 4.5, 6.0 and 12 ml/kg for doses of 0, 12, 30, 45, 60 and 120 mg/kg/day

Study design:

Test article or vehicle (Plasmagelan^{R}) was administered orally (via gavage) on a daily basis starting 2 weeks prior to mating and continued until gestational day 16. Pregnant female rats were scheduled for sacrifice on gestational day 16.

Number/sex/group: 8 females/dose

Parameters and endpoints evaluated:

Toxicity parameters evaluated in this study included mortality (daily), clinical signs (daily), body weights (days 1, 5, 8, 12 and 15 during premating period and daily during the gestation period), food consumption (daily) and gross necropsy (on gestational day 16). The following parameters were measured during the gross necropsy: the number of resorptions, live and dead fetuses, number of implantation sites and number of corpora lutea. In addition, all fetuses were examined for external findings.

The stages of the estrous cycle were checked daily during the 2 week premating period. Vaginal saline rinse samples were collected daily and evaluated on Giemsa stained slides. A regular cycle was defined as 4 days, 4-5 days or 5 days. A shortened irregular cycle was defined as at least one cycle of 2 or 3 days. A prolonged irregular cycle was defined as at least one cycle of 6-10 days. An extended estrus was defined as at least 3 consecutive days of estrus. An acyclic cycle was defined as at least 10 days without estrus or no estrus during the last 9 days of observation.

Blood samples were obtained on the last day of treatment from all treated animals at 0, 0.5, 1, 2, 4 and 6 hours after the last drug administration. ASM 981 levels in blood samples and fetal tissue samples were determined by ———— with a limit of quantification of — ng/ml.

The following reproductive parameters were determined in this study: female mating index/copulation rate (%), female fecundity index/pregnancy rate (%), preimplantation loss (%) and postimplantation loss (%).

Results:

Mortality: No treatment related effects on mortality were noted in this study.

Clinical signs: No treatment related effects on clinical signs were noted in this study.

Body weight: A transient body weight loss was noted during the first 5 days of treatment in the 12, 60 and 120 mg/kg/day dose groups. However, body weight was not affected during the gestational period in all dose groups.

Food consumption: Food consumption was slightly decreased during the first 5 days of treatment only in the 120 mg/kg/day dose group. No treatment related effects on food consumption was noted during the remainder of the study.

Estrus Cycle: Prolongation of the estrous cycle was noted in the 120 mg/kg/day dose group. Two of the eight animals showed a regular 5 day cycle. One rat had a prolonged cycle and three rats were acyclic. Even though eight females were mated in the 120 mg/kg/day dose group, only 6 females were assessable in this dose group (became pregnant).

Gross Pathology: No treatment related macroscopic findings were noted for the female animals in this study. In addition, no treatment related external fetal malformations were noted in this study.

Toxicokinetics: A summary of the toxicokinetic parameters (mean ± SD) is provided in the following table.

Dose (mg/kg/day)	C_{max} (ng/ml)	T_{max} (hr)	$AUC_{0\text{-}6\ hr}$ (ng·hr/ml)	Embryo Conc. (ng/g)	Embryo/ $Plasma_{6\ hr}$ ratio
12	76.5 ± 73.3	0.6 ± 0.2	185 ± 173	11.2 ± 4.7	0.9 ± 0.2
30	282 ± 229	0.9 ± 0.6	917 ± 879	66.1 ± 80.0	1.1 ± 0.5
45	307 ± 186	0.8 ± 0.3	949 ± 709	59.2 ± 63.1	1.0 ± 0.7
60	416 ± 340	1.3 ± 1.4	1136 ± 801	69.6 ± 70.1	1.6 ± 0.9
120	3049 ± 2015	1.2 ± 1.4	13121 ± 9285	--[a]	--[a]

a – no viable fetuses for analysis

An approximate dose dependent increase in ASM 981 systemic exposure was noted for the doses from 12 – 60 mg/kg/day this study. An overproportional increase in systemic exposure was noted in the 120 mg/kg/day dose group. It is important to note that all high dose females showed 100% absorptions. Therefore, there were not viable fetuses in the high dose group for embryo concentration analysis. The study report states that these females could not be considered pregnant. In addition, the study report states that high plasma concentration observed in the high dose animals may be an indicator of slow metabolism in nonpregnant females compared with pregnant females.

The embryo concentrations of ASM 981 increased ~6X from the 12 to 30 mg/kg/day dose level. The embryo concentrations of ASM 981 remained at equivalent levels for the 30, 45 and 60 mg/kg/day dose groups. This may be an indication of maximum placental transfer capacity to the embryo. The concentration ratios for embryonic tissue/maternal plasma at 6 hr ranged from 0.9 – 1.6 over the dose range from 12 – 60 mg/kg/day. This ratio indicated good placental transfer of ASM 981 to the fetal compartment.

Reproduction Data: No treatment related effects on female mating index or female fecundity index was noted in this study. A dose dependent increase in percent post-implantation loss was noted in the 45 (34%), 60 (81%) and 120 (100%) mg/kg/day dose groups compared to control animals (13.5%). A corresponding dose dependent increase in percent resorption was noted in the 45 (28.7%), 60 (76%) and 120 (98.2%) mg/kg/day dose groups compared to control animals (10.9%).

Summary of individual study findings:

A decrease in body weight and food consumption was noted in high dose animals during the first 5 days of the study. Adverse effects on female fertility, an increase in postimplantation loss, was noted at the 45, 60 and 120 mg/kg/day dose levels. The percent postimplantation loss was 100% in the high dose group. This result demonstrates that ASM 981 has embryotoxic potential at dose levels without general maternal toxicity. The NOAEL identified in this study was 30 mg/kg/day ($AUC_{0\text{-}6\ hr}$ = 917 ng·hr/ml; C_{max} = 282 ng/ml). The study report stated that the doses for the main oral fertility and embryo-fetal development studies should be 0, 2, 10 and 45 mg/kg/day ASM 981 based on the results of this study. I concur that this appears to be a reasonable dose range selection for those studies.

Reproductive Toxicology Study #2:

An oral combined fertility and embryo-fetal development study in rats (Drug form: lyophilisate suspension)

Study title: An oral combined fertility and embryo-fetal development study in rats (Drug form: lyophilisate suspension)

Key study findings: No signal for malformations was noted in this study. No effects on paternal toxicity or male reproductive performance were noted in this study. Embryotoxicity and estrus cycle disruption was noted in high dose females.

Study no.:	T-112/203-113
Sandoz Study No.:	3053R
Volume #, and page #:	69, 5-1
Conducting laboratory:	Sandoz Pharma Ltd., Basle, Switzerland
Date of study initiation:	3/6/95
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Drug, and lot#:	ASM 981 – batch# Y289 1094
Formulation/vehicle:	Water & Plasmagelan^R (Note: ASM 981 lyophilisate was reconstituted using water and then further diluted with the plasma volume surrogate Plasmagelan^R to adjust to the final drug concentrations)

Methods:

Species/strain:	Wistar rats; 10 weeks old; males: 307 – 309 grams; females: 190 – 241 grams
Doses employed:	0, 2, 10 and 45 mg/kg/day
Route of administration:	Oral (gavage); Dose volumes = 4.5, 1.0 (diluted solution), 1.0 and 4.5 ml/kg for doses of 0, 2, 10, and 45 mg/kg/day

Study design:

Test article or vehicle (Plasmagelan^{R}) was administered orally (via gavage) on a daily basis for 7 days/week. Male rats were treated for 4 weeks prior to mating and during the 3 week mating period. Female rats were treated for 2 weeks prior to mating until gestation day 16. One male rat from each dose group was mated with one female from the corresponding dose group. The females in the TK satellite groups were mated with control untreated male animals that were not part of the study.

Number/sex/group: 20/sex/dose in main study; 6 females/dose in TK satellite group

Parameters and endpoints evaluated:

Toxicity parameters evaluated in this study included mortality (daily), clinical signs (daily), body weights (daily), food consumption (daily) and gross necropsy. The gross necropsy was conducted for main study females on gestational day 21, for TK satellite females on gestational day 16 and for males on between days 50 – 53 of treatment. During the necropsy for the male animals the weights of testes and epididymides were recorded. A sperm sample was obtained just prior to sacrifice for motility analysis. In addition, one testis was reserved from testicular sperm head count and the other was preserved along with the epididymides for histopathological analysis.

The following parameters were measured during the gross necropsy in females: the number of resorptions, live and dead fetuses, number of implantation sites and number of corpora lutea. In addition, all fetuses were examined for external findings. Examination of the viable F1 fetuses included body weight, placental weights and fetal sex. All fetuses were examined for soft tissue and skeletal abnormalities.

The stages of the estrous cycle were checked daily during the 2 week premating period. Vaginal saline rinse samples were collected daily and evaluated on Giemsa stained slides. A regular cycle was defined as 4 days, 4-5 days or 5 days. A shortened irregular cycle was defined as at least one cycle of 2 or 3 days. A prolonged irregular cycle was defined as at least one cycle of 6-10 days. An extended estrus was defined as at least 3 consecutive days of estrus. An acyclic cycle was defined as at least 10 days without estrus or no estrus during the last 9 days of observation.

After 50 applications of test article, blood samples were obtained from five randomly selected male rats in each dose group at 0.5, 1, 2, 4, 6 and 24 hours after test compound administration. Blood samples were obtained on the last day of treatment from TK satellite females at 0, 0.5, 1, 2, 4 and 6 hours after the last drug administration (gestational day 16). The live embryos were pooled per liter for analysis of test article concentration after the last blood collection point. ASM 981 levels in blood samples and fetal tissue samples were determined by

[redacted] The limit of quantification was [redacted] mg/ml for blood samples and 1 ng/g for embryonic tissue.

The following reproductive parameters were determined in this study: male mating index/copulation rate (%), male fertility index (%), female mating index/copulation rate (%),

female fecundity index/pregnancy rate (%), preimplantation loss (%) and postimplantation loss (%).

Results:

Mortality: No treatment related effects on mortality were noted in this study.

Clinical signs: No treatment related effects on clinical signs were noted in this study.

Body weight: No treatment related effects on body weight were noted in this study.

Food consumption: No treatment related effects on food consumption were noted in this study.

Estrus Cycle: A disturbed estrus cycle was detected in high dose females. The number of females with acyclic cycle was increased in high dose females (4/20) compared to control females (1/20).

Male Gross Pathology: No treatment related effects on testis or epididymide organ weights were noted in this study. No treatment related macroscopic findings were noted in this study.

Female Gross Pathology: No treatment related macroscopic findings were noted in this study.

Toxicokinetics: A summary of the toxicokinetic parameters (mean ± SD) for female satellite animals is provided in the following table.

Dose (mg/kg/day)	C_{max} (ng/ml)	T_{max} (hr)	$AUC_{0\text{-}6\ hr}$ (ng·hr/ml)	Embryo Conc. (ng/g)	Embryo/ $Plasma_{6\ hr}$ ratio
2	8.6 ± 4.0	1.0 ± 1.5	21.0 ± 17.4	0.62 ± 0.70	0.96 ± 1.86
10	112 ± 86	1.4 ± 2.3	194 ± 125	10.0 ± 3.4	0.73 ± 0.30
45	282 ± 43	0.5 ± 0.0	620 ± 149	40.5 ± 18.3	1.09 ± 0.21

Note: $AUC_{0\text{-}6\ hr}$ values were the only values provided in the study report for female animals. However, extrapolated $AUC_{0\text{-}24\ hr}$ values for the 10 and 45 mg/kg/day female dose groups were provided in the label section of the NDA for purposes of calculating multiples of human exposure. The extrapolated $AUC_{0\text{-}24\ hr}$ values for the 10 and 45 mg/kg/day female dose groups were 465 and 1448 ng·hr/ml, respectively.

A dose dependent increase in ASM 981 systemic exposure in pregnant females was noted in this study. The embryo concentrations of ASM 981 increased ~40X over the dose range tested in this study. The concentration ratios for embryonic tissue/maternal plasma at 6 hr ranged between 0.73 ng/g in the low dose group to 1.09 ng/g in the high dose group. These ratio levels indicate good placental transfer of the ASM 981.