CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 21-145

PHARMACOLOGY REVIEW(S)

JUN 20 2000

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

KEY WORDS: Vaniqa, DFMO, eflornithine, female facial histuism

Reviewer Name: Barbara Hill
Division Name: Dermatologic and Dental Drug Products
HFD#: HFD-540
Review Completion Date: 6-13-00

NDA number: 21-145
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Information to sponsor: Yes () No (X)

Sponsor: Westwood-Squibb Colton Holdings Partnership
100 Forest Avenue
Buffalo, NY 14213-1091
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Manufacturer for [redacted]

[redacted]

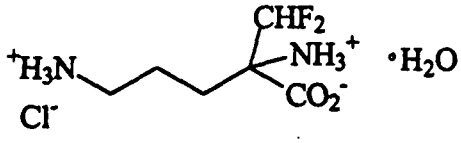
or

[redacted]

Drug:

Code Name: BMS 203522
Generic Name: eflornithine HCl 15% cream, DFMO
Trade Name: Vaniqa
Chemical Name: 2-(difluoromethyl)-DL-ornithine monohydrochloride monohydrate (DFMO)
CAS Registry Number: 96020-91-6
Molecular Formula/ Molecular Weight: $C_6H_{12}F_2N_2O_2 \cdot HCl \cdot H_2O$ / 236.7

Structure:



Relevant INDs/NDAs:

1) NDA 19-879 (Treatment of Trypanosoma Brucei Gambiense Sleeping Sickness, intravenous; HFD-590)
2) IND
3) IND
4) IND

Drug Class: Irreversible inhibitor of ornithine decarboxylase; antineoplastic; antipneumocystis; antiprotozoal (Trypanosoma)

Indication: Hair growth in hirsute women

Clinical formulation:

The composition of the to-be-marketed cream formulation (15%) is provided in the following table:

Ingredient	% w/w	Function
Eflornithine Hydrochloride (BMS 203522)	15.0	Active
Water		
Glyceryl stearate and PEG-100 stearate		
Cetearyl alcohol and ceteareth-20		
Mineral oil, NF		
Stearyl alcohol, NF		
Dimethicone		
Phenoxyethanol		
Methylparaben		
Propylparaben		

Nonclinical formulations:

Note: Several different nonclinical BMS-203522 formulations were used during the drug development process. They are listed below for reference purpose. The SP106A formulation is quite similar to the to-be-marketed formulation described above.

Ingredient (%w/w)	BMS-203522 formulations				Vehicle	
	SP33	SP106A	SP106B	SP106A	SP33V	SP106V
BMS-203522						
Water						
Glyceryl Stearate and PEG-100 Stearate						
Cetearyl Alcohol and Cetereth-20						
Mineral Oil, NF						
Stearyl Alcohol, NF						
Dimethicone						

Dose:

A thin layer of cream is to be applied to the skin above the upper lip or under the chin that contains hair twice a day for up to six months. Human dosing has been estimated to be 2.5 mg/kg/day for a 50 kg female (92.5 mg/m^2/day).

Route of administration: Topical dermal

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

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Review Table of Contents

INTRODUCTION AND DRUG HISTORY:

BMS-203522 (eflornithine or 2-difluoromethylornithine {DFMO}) is an irreversible inhibitor of the enzyme orinithine decarboxylase (ODC). ODC is responsible for the catalysis of ornithine to putrescine. Putrescine and other polyamines (i.e., spermidine and spermine) are present in all living cells and are considered to play an important role in the regulation of cell growth and differentiation. ODC is present in the hair follicle and would be required for hair growth in this tissue. BMS-203522 is an inhibitor of ODC and is being developed as a topical product to reduce the rate of growth of unwanted facial hair in hirsute women.

The initial topical drug formulation for BMS-203522 was initially developed by the Gillette Medical Evaluation Laboratories (GMEL) as a [redacted] (SP33, IND [redacted] and subsequently reformulated to a cream formulation at [redacted] 15% concentrations of BMS-203522 (SP106 formulation series, IND [redacted]. The majority of the initial nonclinical toxicology studies, including single and repeat dose studies up to 26 weeks, were sponsored or conducted by GMEL.

After the completion of the initial IND nonclinical toxicity studies, Bristol-Myers Squibb (BMS) entered into a partnership to develop BMS-203522 for the treatment of female facial hirsutism. Nonclinical toxicity studies performed by BMS included a phototoxicity study in guinea pigs, a 1 year dermal study in miniature swine, dermal studies of embryo-fetal development in rats and rabbits, genotoxicity studies, a 1 year photocarcinogenicity study in hairless mice and a 2 year dermal carcinogenicity study in mice. The 15% BMS-203522 formulation tested by BMS was designated in some studies as a lotion. The sponsor has stated that the vehicle is the same as the SP106 cream formulation except for the concentration of [redacted]

Eflornithine hydrochloride (HCl) has been used for over 15 years as an intravenous injection (Ornidyl) to treat West African (Gambian) trypanosaminasis caused by T.b. gambiense (sleeping sickness). Ornidyl was cleared for marketing for this purpose by the FDA in 199[redacted] (NDA 19-879) and in Europe in 1991. Although not currently marketed in the United States, it is still available in countries where the disease is endemic.

DMFO is being investigated by the National Cancer Institute as a potential oral chemopreventive drug. DMFO is being developed as a chemopreventive agent against cancers with pronounced proliferative phases such as colon, bladder and breast. NCI has sponsored several nonclinical toxicity studies including chronic toxicity, reproductive toxicity and genotoxicity studies. The results of these studies will be summarized below in the following few paragraphs.

Chronic toxicity studies (52 weeks) in rats and dogs were performed by NCI to characterize the toxicities of DMFO at high doses and to support the further development in clinical trials[1]. DMFO was administered by gavage to Charles River CD rats at doses of 400, 800 and 1600 mg/kg/day. Toxicities noted in the mid and high dose groups included weight loss, increased platelets, alopecia and skin abrasions, dermatitis, liver necrosis and gastric inflammation. The NOEL in this study was 400 mg/kg/day. DMFO was administered via capsule to dogs at doses of 50, 100 and 200 mg/kg/day. Toxicities noted in all dose groups included conjunctivitis, hyperkeratosis and alopecia, and cystic intestinal crypts. A NOEL could not be determined in this study.

Target organs of toxicity for DFMO treatment identified in these nonclinical oral chronic toxicity studies include skin, liver, gastrointestinal tract and conjuctiva. It is interesting to note

[1] Crowell JA, Goldenthal EI, Kelloff GJ, Malone WF and Boone CW (1994) Chronic toxicity studies of the potential cancer preventive 2-(difluoromethyl)-*dl*-ornithine. Fundam. Appl. Toxicol. 22: 341-354.

that oral DMFO side effects in humans include seizures, anoxia, gastrointestianal symptoms, anemia, thrombocytopenia and decreased hearing acuity. The thrombocytopenia and ototoxicity effects have not been noted in oral nonclinical toxicity studies to date.

Some genotoxicity tests that have been conducted with DFMO are mentioned in an article included in the submission[2]. Unfortunately, not much detail was provided about these genotoxicity studies in the article. It would have more helpful if the sponsor had actually submitted the study reports for these genotoxicity studies with the NDA submission. For reference purposes, I have reproduced the section from the paper that discusses the gentoxicity studies and results below.

"DFMO did not significantly increase SCE frequency in CHO cells *in vitro* or micronucleated cell frequency in bone marrow of mice treated *in vivo*. DFMO was also negative in the Ames mutagenicity assay in Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537."

Oral reproductive toxicity studies for DMFO have been contracted for by NCI. Embryotoxic, fetotoxic and teratogenic potential were evaluated in CD rats and New Zealand White rabbits[3]. DFMO was administered daily to time mated rats and rabbits via gavage during the period of organogenesis. Pregnant rats received 0, 30, 80 or 200 mg/kg/day DMFO during gestations days 6 – 17. Fetuses were obtained by cesarean section on gestation day 20. Fetal toxicity in the absence of maternal toxicity was observed at 200 mg/kg/day as significantly decreased fetal body weights and increased incidence of litters with skeletal variations (e.g. 14th rudimentary rib, 14th full rib and/or 27th presacral vertebrae). No treatment related fetal skeletal malformations or external or visceral anomalites were noted at any dose level in this study. The maternal NOEL in rats was 200 mg/kg/day and the fetal NOEL in rats was 80 mg/kg/day.

Pregnant rabbits received 0, 15, 45 or 135 mg/kg/day DMFO during gestation days 7 – 20. Fetal toxicity in the absence of maternal toxicity was observed at 135 mg/kg/day as significantly decreased fetal body weights. No treatment related fetal external, visceral or skeletal anomalies were noted at any dose level in this study. The maternal NOEL in rabbits was 135 mg/kg/day and the fetal NOEL in rabbits was 45 mg/kg/day.

The sponsor included information on potential impurities in the drug product within the chemistry portion of the NDA submission. Ernie Pappas, chemistry reviewer, provided me with copies of the impurity information contained in the chemistry section. A brief review of this information will be provided in the following few paragraphs.

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[2] Kelloff GJ, Crowell JA, Boone CS, Steele VE, Lubert RA, Greenwald P, Alberts DS, Covey JM, Doody LA, Knapp GG, at al. (1994) Clinical development plan: 2-difluoromethylornithine (DMFO). J. Cell Biochem. Suppl. 20: 147-165.

[3] Kirchner DL, Levine BS, Mercieca MD and Crowell. (1997) Oral developmental toxicity studies of α-difluoromethylornithine (DFMO) in rats and rabbits. Toxicologist 36: 259.

The sponsor states that there are no unknown inpurities present at a level above — in the 15% BMS 203522 lotion. The sponsor lists the following impurities that are present in the drug product at a level of less than — The sponsor states that all impurities can be detected by an analytical impurity profile method.

Chemical Name	Internal Code

[a] – identified as the product of the

[b] – identified as a

[c] – identified as a

[d] – identified as a

It appears that the sponsor has an adequate analytical method to be able to detect these impurities in the drug product and will maintain each one below the — level. This is acceptable and no additional nonclinical toxicity studies for these impurities are required at this time.

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STUDIES REVIEWED WITHIN THIS SUBMISSION:

Nonclinical Pharmacology Studies:

1) In-vitro penetration of DFMO in guinea pig skin (GMEA 7766)
2) A sensitive ——— assay to measure polyamine inhibitors in the hamster flank organ model (RRCR 2880; 258)
3) Inhibition of hair mass in hamster flank organ (GMEA 7764)
4) Growth rate of hamster flank organ hairs (SR-950034)
5) Inhibition of hair growth by dl-α-Difloromethyl Ornithine (ODC) (BMS-X. —)
6) Inhibition of hair growth by dl-α-Difloromethylornithine (DMFO) (RRCF 2835)

Nonclinical Pharmacokinetic Studies:

1) Absorption, excretion and tissue distribution of radioactivity in mice following a single dermal dose of [^{14}C]BMS-203522 in SP106A (GMEA 7810; BMS 920000132)
2) Effectiveness of dermal tape stripping to determine percutaneous absorption and preliminary pharmacokinetics of [^{14}C]BMS-203522 in rats following a single or twice-daily dermal doses and single oral doses (GMEA 7801; BMS 920000133)
3) Absorption, excretion, and tissue distribution of radioactivity in female rats following twice-daily dermal and once-daily oral doses of [^{14}C]BMS-203522 administered for 7 days (GMEA 7807; BMS 910000135)
4) Summary of the *in vitro* skin permeation profile of BMS-203522-1 (eflornithine) from different topical formulations during product development (CHOU-TW-99009)

Acute Toxicology Studies:

1) Acute dermal toxicity study in rabbits – limit test (GMEA 7597; 744-459)
2) Acute oral toxicity study of SP106A in rats – limit test (GMEA 7765; — 10403309)

Special Toxicology Studies:

1) Primary eye irritation study in rabbits (GMEA 7597; 744-456/744-457)
2) Primary eye irritation study of SP106A in rabbits – unwashed eye (GMEA 7765; — 10403310)
3) Primary eye irritation study of SP106A in rabbits – washed eye (GMEA 7765; 10403311)
4) Guinea pig sensitization test (Buehler's technique modified) (GMEA 7597; 744-460)
5) Phototoxicity study in Guinea pigs (97687)

Repeat Dose Dermal Irritation Studies:

1) Repeat application dermal effect study in hamsters (— 744-573)
2) Repeat insult rabbit skin irritation study (GMEA 7597; 744-458)

3) Repeat insult rabbit skin irritation study of samples SP106A, SP106B, SP106C and SP106V – (5 day repeat) (GMEA 7765; — 10403312)
4) Repeat insult rabbit skin irritation study of samples SP106A, SP106B, SP106C and SP106V – (14 day repeat) (GMEA 7768)
5) 14-day dermal irritation study in rabbits (GMEA 7788)
6) 21-day repeat insult skin irritation study of SP106 formulations in rabbits (GMEA 7781)

Repeat Dose Dermal Toxicology Studies:

1) 14-day dermal exploratory toxicity study in hairless mice (97659)
2) 13-week dermal toxicology study in mice (GMEA 7808)
3) 26-week dermal toxicity study with SP106V, SP106A, SP33 and SP33V in rats (GMEA 7793; 744-576)
4) Dermal toxicology study with SP106V, SP106A and SP106C in rabbits (GMEA 7783)
5) 15% BMS-203522 lotion: One year dermal toxicity study in miniature swine (97631)

Reproductive Toxicology Studies:

1) 15% BMS-203522 lotion: dermal study of fertility and early embryonic development in rats (96676)
2) Developmental toxicity study in rats (— 744-578)
3) 15% BMS-203522 lotion: dermal study of embryo-fetal development in rats (96014)
4) 15% BMS-2-3522 lotion: dermal study of embryo-fetal development in rabbits (96677)
5) Perinatal and postnatal study with eflornithine hydrochloride in rats (T-86-20)

Genotoxicity Studies:

1) BMS 203522 ames reverse-mutation study in *Salmonella* and *Escherichia Coli* (97647)
2) BMS 203522 cytogenetics study in primary human lymphocytes (97651)
3) 15% BMS 203522 lotion: dermal micronucleus study in rats (97654)

Carcinogenicity Studies:

1) Twelve-month photocarcinogenicity study in hairless mice (97719)
2) Two-year dermal carcinogenicity study in mice (96701)

PHARMACOLOGY:

Background Information: Eflornithine was evaluated in several vehicles for topical activity in the hamster flank organ and the mouse models. The different vehicles are listed below.

a) **Vehicle** (H_2O)
b) **SP33 vehicle** (H_2O,

c) **SP106 vehicle** (H_2O, glyceryl stearate and PEG-100 stearate, cetearyl alcohol and ceteareth-20, mineral oil, stearyl alcohol, [redacted], phenoxyethanol, methylparaben, [redacted])

In addition, it is important to note that SP33 represents [redacted] BMS-203522 in the SP33 vehicle. SP106A, B, C and D represent 15%, [redacted] BMS-203522, respectively, in the SP106 vehicle. SP106V represents the vehicle only.

The flank organs (costovertebral glands) of the Golden Syrian hamster are used as a model for the human sebaceous gland. The flank organs are grossly visible paired structures, situated on the back of the animal that contain sebaceous glands, large pigmented hair follicles and dermal melanocytes. The hamster flank organ model was the primary animal model used for evaluating the potential efficacy of BMS-20355 as a treatment for hirsutism. The design of the following studies compared hair growth on treated versus untreated flank organs within individual animals. The male Golden Syrian hamsters used in these studies were 9-10 weeks old at the start of the experiments and were housed individually in wire rack suspended cages.

Two *in vivo* nonclinical pharmacology studies used C_3H mice that were in the second telogen or resting phase of hair growth. The C_3H mouse is a useful model for studying hair growth. The reason for this is that skin pigmentation for this mouse is provided by the melanocytes of the hair follicle and not the epidermis. In the telogen or resting phase of the hair follicle the skin is pink. One of the first visual signs of anagen or the growth phase is graying of the skin. As the anagen phase progresses, the skin becomes darker in color leading to appearance of the hair fiber at the skin surface. These visual changes were utilized in this animal model as an *in vivo* assay to assess drug effect on anagen induction or inhibition.

In this *in vivo* animal model, the dorsal hair was clipped and mice revealing pink skin were selected to screen for suppression of hair growth. The hair over a 2 x 3 cm area was depilated to induce synchronized hair follicle stimulation or the anagen phase. In this model, agents that inhibit hair growth will retard or prevent hyper-pigmentation of skin and subsequent hair growth. On day 3 after depilation, test compounds were applied 1x or 2x daily, 5 days a week for up to 2 weeks. The test sites were monitored for changes in skin pigmentation, new hair growth and density of hair growth during this period.

Mechanism of Action:

BMS-203522 (eflornithine or 2-difluoromethylornithine {DFMO}) is an irreversible inhibitor of the enzyme orinithine decarboxylase (ODC). ODC is responsible for the catalysis of ornithine to putrescine. Putrescine and other polyamines (i.e., spermidine and spermine) are present in all living cells and are considered to play an important role in the regulation of cell growth and differentiation. ODC is present in the hair follicle and would be required for hair growth in this tissue. BMS-203522 is an inhibitor of ODC and is being developed as a topical product to reduce the rate of growth of unwanted facial hair in hirsute women.

Drug Activity Related to Proposed Indication:

Drug Activity Study #1:

A sensitive ——— assay to measure polyamine inhibitors in the hamster flank organ model

Study Title:	A sensitive ——— assay to measure polyamine inhibitors in the hamster flank organ model
Study No:	RRCR 2880; 258
Conducting laboratory:	Gillette Research Institute
Date of study:	Study conducted between June 1988 and April 1990
GLP compliance:	No

Drug Activity Study #2:

Inhibition of hair mass in hamster flank organ

Study Title:	Inhibition of hair mass in hamster flank organ
Study No:	GMEA 7764
Conducting laboratory:	Gillette Medical Evaluation Laboratories

Date of study: Study conducted between April and December, 1991
GLP compliance: No

The objective of this study was to evaluate the dose response profile of quantitative hair mass inhibition and perceptible decrease in hair growth following topical application of BMS-203522 in various test formulations. Hamsters were treated (Mon-Fri; 13 treatments over 18 day period) with BMS-203522 in various vehicle formulations or vehicle only on the left flank organs. The contralateral vehicle treated right flank organs served as controls. The treatments are provided in the following table.

Study Design

Group	Number of Animals	Left Flank Organ	Right Flank Organ
1	15	SP106V (Vehicle)	SP106V
2	15	SP106A (15% BMS-203522)	SP106V
3	15	SP106B — BMS-203522)	SP106V
4	15	SP106C — BMS-203522)	SP106V
5	15	SP106D — BMS-203522)	SP106V
6	15	SP33 — BMS-203522)	SP33V

The results from this study are provided in the following table.

Flank Organ Hair Mass Values

Treatment	Flank Organ Hair Mass (mean ± SEM)		
	Treated (mg)	Vehicle (mg)	% Inhibition
SP106V	1.96 ±0.16	1.96 ± 0.14	-1.48 ± 5.13
SP106A	0.28 ± 0.04	1.80 ± 0.11	84.22 ± 2.08
SP106B	0.20 ± 0.04	1.59 ± 0.08	87.64 ± 2.41
SP106C	0.32 ± 0.06	2.03 ± 0.24	84.51 ± 2.56
SP106D	0.68 ± 0.08	1.87 ± 0.19	59.96 ± 5.24
SP33	0.70 ± 0.06	2.32 ± 0.14	68.26 ± 2.80

Treatment with a ——————15% dose of BMS-20355 in the SP106 vehicle produced equal degrees of hair mass inhibition of ~85%. This was significantly higher that the level of hair mass inhibition (~60%) exhibited by the —— BMS-203522 in SP106 vehicle. The level of hair mass inhibition (~68%) after a — BMS-203522 dose in the SP33 vehicle was significantly less than seen after treatment with SP106A, B or C. No evidence of contralateral hair mass inhibition of untreated flank organs was noted in the study.

Drug Activity Study #3:

Growth rate of hamster flank organ hairs

Study Title:	Growth rate of hamster flank organ hairs
Study No:	SR-950034
Conducting laboratory:	Gillette Research Institute
Date of study:	Report dated December 21, 1995
GLP compliance:	No

Two similar studies were conducted with male Golden Syrian hamsters (10-13 weeks old) in this report to evaluate the effects of the topical formulations SP33, SP106A and SP106B on hamster flank organ hair growth rate. The hamster flank organs were chemically depilated (Study 1) or plucked with a curved forceps (Study 2) prior to the first treatment. Treatments were administered Monday through Friday. Test article (10 mg) was administered to the left flank organ and the corresponding vehicle was administered to the right flank organ. The _____ System was used to determine the hair length of plucked hamster flank organ hairs.

Study 1 was an 18 day study in which the same animal was repeatedly plucked with curved tip forceps in a different location on the flank organ. Flank organs were chemically depilated at day 0 prior to treatment. Hair plucking from the flank organ occurred on days 3, 7, 10, 14 and 17. The treatments for Study 1 are provided in the following table.

Study 1 Design

Group	No. of Animals	Left Flank Organ	Right Flank Organ
1	8	SP106B (15% eflornithine)	SP106V
2	8	SP33 (15% eflornithine)	SP33V
3	8	SP33V (vehicle)	SP33V

Study 2 was designed to assess changes in growth rates over seven day intervals following progressively longer treatment duration's of one, two or four weeks. All hairs were completely removed from both flank organs of each hamster by mechanical epilation (plucking with curved forceps) performed on days 0, 7, 14 and 28. This study designed provided three 7 day growth rates comprising days 0-7, 8-14 and 21-28 following varying treatments of one, two and four weeks duration. The treatments for Study 2 are provided in the following table.

Study 2 Design

Group	No. of Animals	Left Flank Organ	Right Flank Organ
1	8	SP106A (15% eflornithine)	SP106V
2	7	SP106V	No treatment

The results from Study 1 are provided in the following table.

Study 1 Results (mean ± St. Dev.)

Treatment	Left Flank Organ		Treatment	Right Flank Organ	
	Day	Hair Length (mm)		Day	Hair Length (mm)
SP33	3	2.43 ± 0.15	SP33V	3	2.50 ± 0.15
	7	2.67 ± 0.25		7	3.87 ± 0.40
	10	2.50 ± 0.23		10	4.66 ± 0.77
	14	2.42 ± 0.59		14	5.66 ± 0.59
	17	1.63 ± 0.26		17	7.89 ± 0.61
SP106B	3	2.38 ± 0.38	SP106V	3	2.37 ± 0.40
	7	2.37 ± 0.26		7	3.86 ± 0.56
	10	2.18 ± 0.23		10	3.78 ± 1.17
	14	2.02 ± 0.18		14	4.20 ± 1.15
	17	1.31 ± 0.23		17	5.47 ± 0.81
SP33V	3	2.57 ± 0.17	SP33V	3	2.60 ± 0.13
	7	4.19 ± 0.42		7	4.18 ± 0.42
	10	5.11 ± 0.99		10	5.10 ± 0.85
	14	5.64 ± 0.38		14	5.84 ± 0.98
	17	8.28 ± 0.53		17	8.21 ± 0.90

Treatment with eflornithine in either the SP33 or SP106B topical formulation caused a 30-40% decrease in hair length compared to vehicle by day 7 and a 65-80% decreased by day 14.

The results from Study 2 are provided in the following table. All hair length measures correspond to seven day intervals.

Study 2 Results (mean ± SEM)

Treatment	Left Flank Organ		Treatment	Right Flank Organ	
	Day	Hair Length (mm)		Day	Hair Length (mm)
SP106A	7	2.71 ± 0.08	SP106V	7	4.50 ± 0.15
	14	1.68 ± 0.09		14	4.83 ± 0.19
	28	1.59 ± 0.07		28	4.52 ± 0.13
SP106V	7	4.89 ± 0.08	No treatment	7	5.31 ± 0.10
	14	4.48 ± 0.09		14	5.32 ± 0.11
	28	4.46 ± 0.11		28	5.69 ± 0.08

Hair growth rats were reduced 43% during the first week of 15% eflornithine (SP106A) treatment and 63% and 64% after two and four weeks of treatment, respectively, compared to

contralateral vehicle treated controls. These results suggest that the initial effect on hair growth begins during week one with further enhancement during week 2, which is maintained through week 4. A slight inhibition of hair growth was noted when comparing control SP106 vehicle to untreated controls. The contract lab states that this may have been due the mechanical forces applied to the hair shaft during application of the test article, which could lead to a weakening of the hair shaft.

Drug Activity Study #4:

Inhibition of hair growth by dl-α-Difluoromethyl Ornithine (ODC)

Study Title:	Inhibition of hair growth by dl-α-Difluoromethyl Ornithine (ODC)
Study No:	BMS-X. —
Conducting laboratory:	—
Date of study:	Report dated December 14, 1998
GLP compliance:	No

Two experiments in C_3H mice in their second telogen phase of hair growth were conducted in this study (10 animals/group). Animals were age matched at 42 days old, which is the onset of the second telogen phase. In the first experiment, 50 µl of vehicle, ——15% eflornithine were applied to clipped areas of the dorsal skin twice daily, 5 days a week for two weeks. In the second experiment, 50 µl of vehicle, ————15% eflornithine was applied twice daily, 5 days a week for two weeks. The vehicle was a cream formula that contained the following ingredients: ceterayl alcohol, ceteareth-20, dimethicone, glyceryl stearate, methylparaben, mineral oil, PEG — stearate, phenoxylethanol, proplparaben, sterayl alcohol and water.

Suppression of hair growth was monitored by changes in skin pigmentation. The grading scale used was the following. Skin color: 0 = normal pink color, 1 = patchy light gray color, 2 = even light gray, 3 = moderate gray, 4 = dark gray. Hair growth and density: 0 = no hair, 1 = first appearance of hair, 2 = patchy hair growth and density, 3 = even hair growth and density, 4 = complete hair growth and density.

The results of experiment 1 show that eflornithine at ——15% effectively retarded the progression of hair growth compared to vehicle. All applications were stopped on day 10 when complete hair growth was noted in the vehicle group. At that time point, a 25% and 55% reduction in hair growth were noted in the ——15% eflornithine treated groups, respectively.

A dose dependent decrease was noted in hair growth in experiment 2. Animals treated with the vehicle and — eflornithine showed complete hair growth. Groups treated with — eflornithine showed signs of diminished hair growth as compared to vehicle treated animals. Very little to no hair growth was apparent over the test sites for animals treated with ——15% eflornithine.

Drug Activity Study #5:

Inhibition of hair growth by dl-α-Difluoromethylornithine (DMFO)

Study Title:	Inhibition of hair growth by dl-α-Difluoromethylornithine (DMFO)
Study No:	RRCF 2835
Conducting laboratory:	The Gillette Company
Date of study:	Study conducted between April, 1985 and December, 1986
GLP compliance:	No

The objective of this study was to determine the ability of topically applied eflornithine to inhibit ODC in the hamster flank organ. Eflornithine in ———, eflornithine in SP33 vehicle or vehicle alone was applied twice to hamster flank organs in 24 hours. The follicle bulbs from the hamster flank organs were excised, minced, homogenized and centrifuged at low speed (12,000 g for 15 minutes). ODC was assayed in the using a ——— assay to measure newly synthesized putrescine directly in biological samples. The procedure for this is the following.

[ ]

The results from this study are presented in the following table (mean ± SEM; n = 6 per treatment).

Treatment	ODC activity in left flank organ	Treatment	ODC activity in right flank organ
500 mg eflornithine in SP33 vehicle	295 ± 100	SP33 vehicle	745 ± 165
500 mg eflornithine in ———	215 ± 45	———	840 ± 225
SP33 vehicle	700 ± 115	SP33 vehicle	765 ± 115

- ODC activity in picomoles putrescine formed per follicle/4 hours

The results show that ODC activity was significantly decreased in eflornithine treated flank organ hair follicles compared to either vehicle control. Therefore, eflornithine in either formulation was able to penetrate into the hair follicle and inhibit ODC activity.

Summary of Pharmacology:

Eflornithine was shown to inhibit tissue ornithine decarboxylase, a key enzyme for the control of cell division. Nonclinical pharmacology studies demonstrated that eflornithine inhibits hair growth in two species in various vehicles and in a dose-dependent manner. The hamster and mouse modes were selected as relevant animal models since they show hormone and hair cycle responses to hair growth. The endpoint, hair mass, is the key clinical target selected for the development eflornithine in this NDA.

PHARMACOKINETICS/TOXICOKINETICS:

Absorption, Distribution and Excretion:

Absorption, Distribution and Excretion Study #1:

Absorption, excretion and tissue distribution of radioactivity in mice following a single dermal dose of [^{14}C]BMS-203522 in SP106A

Study Title:	Absorption, excretion and tissue distribution of radioactivity in mice following a single dermal dose of [^{14}C]BMS-203522 in SP106A
Study No:	GMEA 7810; BMS 920000132
Conducting laboratory:	Bristol-Myers Squibb Company, Pharmaceutical Research Institute, Department of Metabolism and Pharmacokinetics
Date of study:	Final report dated July 1999
GLP compliance:	No

The absorption, excretion and tissue distribution of radioactivity in male and female mice was studied after a single dermal dose of [^{14}C]BMS-203522 (~600 mg/kg), applied as 100 µl of the 15% cream based formulation (SP106A) to each mouse. This study was done in three groups of six male and six female mice each. The doses were removed at 4 hr after application in group 1, and at 6 hr after application for group 2 and 3 mice. Mice in groups 1, 2 and 3 were sacrificed at 4, 24 and 96 hr after dose application, respectively. Blood and selected tissues were collected at the time of sacrifice. Blood was also collected at 8 hr and 12 hr post dose from group 3 mice. Urine and feces were collected at 24 hr intervals or until scheduled sacrifice.

The mean values for radioactivity in urine, feces and tissues is provided in the following table.

Parameter	Male			Female		
	Group 1 (4 hr)	Group 2 (24 hr)	Group 3 (96 hr)	Group 1 (4 hr)	Group 2 (24 hr)	Group 3 (96 hr)
Recovery in urine (% of dose)	0.11 (0.09)	0.21 (0.12)	0.40 (0.31)	0.11 (0.20)	0.43 (0.37)	0.44 (0.26)
Recovery in Feces (% of dose)	0.01 (0.02)	0.16 (0.17)	0.39 (0.20)	<0.005	0.05 (0.03)	0.37 (0.34)
Recovery in Tissues[a] (% of dose)	0.02 (0.02)	0.03 (0.04)	0.01 (0.02)	0.02 (0.01)	<0.005	0.02 (0.05)
Absorption[b] (% of dose)	0.15 (0.07)	0.40 (0.27)	0.77 (0.54)	0.12 (0.20)	0.49 (0.36)	0.84 (0.35)
Recovery in Skin Wash (% of dose)	80.9 (4.41)	73.0 (12.3)	84.0 (3.63)	84.7 (6.41)	82.2 (4.77)	84.8 (9.09)
Recovery in skin[c] (% of dose)	0.009 (0.12)	0.03 (0.03)	0.02 (0.01)	0.13 (0.11)	0.03 (0.02)	0.02 (0.01)
Skin Concentration[d] (µg-equivalent/g)	217 (289)	27.3 (270)	3.62 (6.16)	206 (180)	25.0 (14.8)	5.18 (9.97)
Blood Concentration (µg-equivalent/g)	0.194 (0.113)	0.012 (0.030)	ND	0.194 (0.137)	0.037 (0.029)	ND

[a] – combined recovery in all excised tissues (excluding skin at application site), organs, and carcass at the time of sacrifice

[b] – absorption estimated by the total recovery of radioactivity in urine, feces, tissues (excluding application site), organs, and carcass at the time of sacrifice

[c] – tape stripping (i.e., stratum corneum) at the application site after dose removal by skin wash

[d] – biopsy at the application site

ND – not detectable

The mean recovery in urine and feces ranged from 0.11- 0.44% and <0.005-0.39%, respectively. The mean total recovery of radioactivity in all tissues/organs and the carcass (at the time of sacrifice) ranged from <0.005-0.04% of the applied dose. The mean dermal absorption of BMS-203522 in mice (calculated from the radioactivity recovered in blood, tissues, urine, and feces) was less than 0.84% of the applied radioactive dose for all groups and sexes.

The mean total recovery of radioactivity in urine, feces, tissues, carcass, and wash from the application site averaged 84.2% of the applied dose for all groups. No apparent differences in the excretion, absorption and distribution of radioactivity were noted in male and female mice following single dermal doses of [^{14}C]BMS-203522 in the SP106A formulation.

Absorption, Distribution and Excretion Study #2:

Effectiveness of dermal tape stripping to determine percutaneous absorption and preliminary pharmacokinetics of [^{14}C]BMS-203522 in rats following a single or twice-daily dermal doses and single oral doses

Study Title:	Effectiveness of dermal tape stripping to determine percutaneous absorption and preliminary pharmacokinetics of [^{14}C]BMS-203522 in rats following a single or twice-daily dermal doses and single oral doses
Study No:	GMEA 7801; BMS 920000133
Conducting laboratory:	Bristol-Myers Squibb Company, Pharmaceutical Research Institute, Department of Metabolism and Pharmacokinetics
Date of study:	Final report dated June 1999
GLP compliance:	No

The objective of this study was to determine the effectiveness of dermal tape stripping in determining the dermal penetration of applied doses and to evaluate the absorption, excretion and distribution of radioactivity related to BMS-203522 after dermal and oral doses of BMS-203522 to rats. In group 1, five female rats received a single 50 µl/rat (~50 mg/kg) topical dose of SP106A formulation of [^{14}C]BMS203522. In group 2, five male and 5 female rats received two 50 µl/rat (~100 mg/kg) topical doses of the SP106A formulation, with a 6 hr period between doses. In group 3, five male and five female rats received a single 800 mg/kg oral dose of [^{14}C]BMS-203522 in solution. The group 1 rats were sacrificed at 3 minutes after dose application. The rats in groups 2 and 3 were sacrificed at 24 hours post-dose. The stratum corneum layers of the skin at the application site (groups 1 and 2) and shaved abdominal area (group 3) were removed by the tape-stripping technique. Blood samples from group 2 (first dose for group 2) and group 3 rats were collected at 1, 2, 4, 6, 7, 8, 12 and 24 hours post-dose. Urine and feces from the rats in groups 2 and 3 were collected over 24 hours post-doses and selected tissues and organs (liver, kidneys, spleen and skin) were sampled for radioactivity concentrations at the time of sacrifice.

Radioactivity in blood was generally undetectable after dermal doses. For oral doses, the C_{max} values after oral doses (43.2 and 62.5 µg equivalents/ml in males and females, respectively) occurred within 2 hr post-dose. For animals that received dermal doses, greater than 95% of the applied dose was recovered in the skin washes. After dermal application, the tape stripping (i.e., stratum corneum) and the remaining skin at the test site contained 0.05% and 0.03% of the applied dose in group 1 rats, respectively, and 0.16% and 0.05-0.12% of the dose in group 2 rats, respectively. After oral administration, <0.01% of the dose was recovered in the skin and in the tape strippings. The urinary recovery of radioactivity was 0.04% and 0.03% of the dermal dose in males and females, respectively. Less than 0.01% of the dermal dose was recovered in blood, tissues (excluding skin) or feces of both genders. For the orally dosed animals, the urinary recovery of radioactivity was 23.4% and 26.8% and the fecal recovery was 59.2% and 49.5% in males and females, respectively. Less than 0.01% of the oral radioactive dose was found in the

skin, tape stripping, blood, kidneys, and spleen of both males and females. A small fraction of the oral radioactive dose of [^{14}C]BMS 203522 (0.05% for males and 0.03% for females) was found in the liver.

Absorption, Distribution and Excretion Study #3:

Absorption, excretion, and tissue distribution of radioactivity in female rats following twice-daily dermal and once-daily oral doses of [^{14}C]BMS-203522 administered for 7 days

Study Title:	Absorption, excretion, and tissue distribution of radioactivity in female rats following twice-daily dermal and once-daily oral doses of [^{14}C]BMS-203522 administered for 7 days
Study No:	GMEA 7807; BMS 910000135
Conducting laboratory:	Bristol-Myers Squibb Company, Pharmaceutical Research Institute, Department of Metabolism and Pharmacokinetics
Date of study:	Final report dated July 1999
GLP compliance:	No

The absorption, excretion, and distribution of total radioactivity was studied in nine groups of eight female rats/group. Groups 1-6 received twice daily dermal doses (~30 mg/kg/dose) and groups 7-9 received once daily oral doses (~800 mg/kg/dose) of [^{14}C]BMS203522 for up to 7 days. The oral doses were administered as an 80 mg/ml solution of [^{14}C]BMS203522 and the dermal doses were applied as 50 µl of the 15% topical formulation of [^{14}C]BMS203522 (SP106A formulation). Six hours after topical application of the first daily dose, the non-occulsive cover from the dosing site was removed and the second dose was applied at the dosing site and the cover was replaced. The skin was washed with a gauze pad moistened with water and blotted with a dry gauze pad before the first daily dose application on each day. Rats in groups 1, 2 and 3 were sacrificed at 18 hr after the second dose on day 1, 3 and 5, respectively. The rats in group 4 were sacrificed at 4 hr after the first dose on day 7. Rats in groups 5 and 6 were sacrificed at 18 and 90 hr after the second dose on day 7, respectively. Rats in groups 7, 8 and 9 were sacrificed at 4, 14 and 96 hr, respectively, after the daily oral dose on day 7. Blood and selected tissues were collected at 24 hr intervals. Radioactivity concentrations were determined by [redacted]

The mean concentrations of radioactivity in various tissues and organs are summarized in the following figure scanned from the NDA submission.

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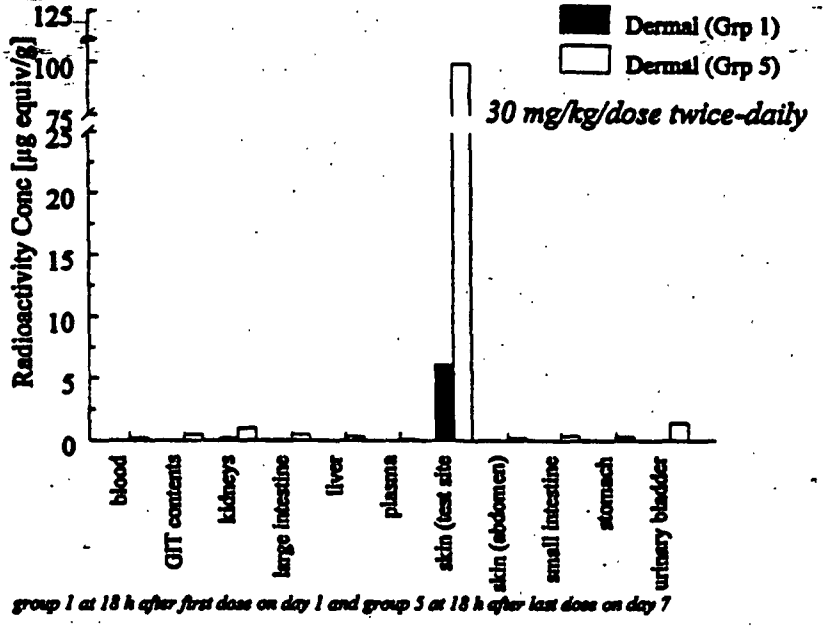
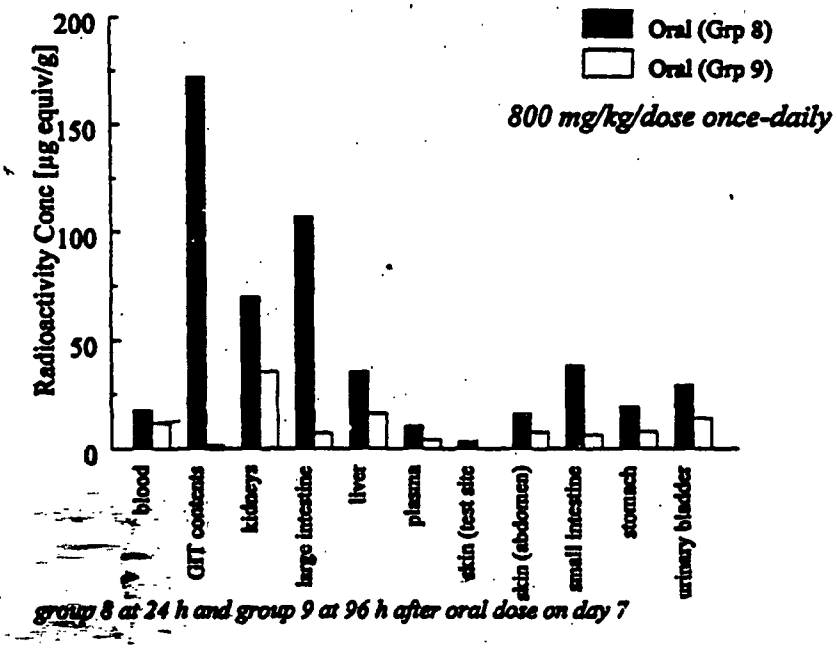
Figure 4 **Concentrations of BMS-203522 equivalents of radioactivity in selected tissues of female rats after twice-daily dermal and once-daily oral doses of [^{14}C]BMS-203522 in study GMEA 7807**



group 1 at 18 h after first dose on day 1 and group 5 at 18 h after last dose on day 7



group 8 at 24 h and group 9 at 96 h after oral dose on day 7

After the application site was washed at the time of sacrifice of the rats, the mean concentrations of radioactivity in skin at the site of application in groups 1-6 were 0.8, 3.4, 6.8, 25.6, 14.2 and 20.9 µg-equivalents of BMS-203522/cm^2, respectively. Concentration of radioactivity in the skin at the application site increased throughout the 7 day dermal dosing

regimen, but decreased when the dosing was discontinued. After both dermal and oral doses of [^{14}C]BMS 203522 in rats, the highest concentrations of radioactivity were generally observed in the tissues and organs associated with dose administration and elimination, but preferential accumulation did not appear to occur in any tissue.

At 1, 2, 3 and 4 hr after oral dosing on day 7 (group 7), the mean blood concentrations of radioactivity were 97.7, 116, 88.0 and 68.2 µg-equivalents/g, respectively. At 24 hr (group 8) and 96 hr (group 9) after the oral dose on day 7, the mean blood concentrations of radioactivity were 17.6 and 11.6 µg-equivalents, respectively.

The extent of urinary and fecal excretion of radioactivity in each group is summarized in the following table.

Parameter	Mean (SD) Radioactivity Data								
	Dermal Dose (30 mg/kg/dose)						Oral Dose (800 mg/kg/dose)		
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Recovery in Urine [% of dose][a]	0.18 (0.10)	0.33 (0.08)	0.70 (0.42)	2.52 (0.91)	2.56 (1.52)	3.31 (1.12)	32.6 (5.60)	35.4 (8.25)	37.7 (4.17)
Recovery in Feces [% of dose][a]	<0.005	0.02 (0.01)	0.05 (0.03)	0.33 (0.15)	0.19 (0.11)	0.57 (0.55)	42.11 (4.11)	53.1 (5.38)	53.5 (3.96)
Recovery in Blood [% of dose]	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Recovery in Tissues[b] [% of dose]	<0.005	NE	NE	NE	0.29 (0.08)	NE	NE	0.69 (0.55)	0.02 (0.01)
Dermal Absorption[c] [% of dose]	0.18 (0.10)	0.35 (0.08)	0.75 (0.45)	2.85 (0.99)	3.04 (1.70)	3.88 (1.36)	NA	NA	NA
Recovery in Skin Wash [% of dose]	97.8 (2.06)	97.2 (1.81)	77.2 (5.12)	80.9 (3.22)	85.9 (2.30)	82.9 (2.70)	NA	NA	NA
Recovery in Skin[d] [% of dose]	0.01 (0.01)	0.01 (0.00)	0.01 (0.00)	0.03 (0.01)	0.01 (0.01)	0.02 (0.03)	NA	NA	NA

[a] – urine and feces were collected up to the time of sacrifice
[b] – combined recovery in all excised tissues (excluding skin at application site), organs, and carcass at the time of sacrifice
[c] – absorption estimated by the total recovery of radioactivity in urine, feces, tissues (groups 1 and 5 only), and blood at the time of sacrifice
[d] – application site
NE – not evaluated
NA – not applicable

The extent of urinary and fecal excretion of radioactivity in rats, following oral administration of [^{14}C]BMS-203522, was 32-38% and 42-54%, respectively. After twice daily dermal application of [14C]BMS-203522 to rats for 5 days, the urinary recovery of radioactivity increased from 0.18% to 0.70% and the fecal recovery increased from <0.005% to 0.05%.

The mean total recovery of radioactivity excreted in urine and feces (including radioactivity in the washes from application site for dermal doses) averaged 90.2% of the oral doses and 91.1% of the dermal doses for all groups. The dermal absorption of BMS-203522 ranged from 0.18% to 0.75% (groups 1, 2 and 3) based on recovery of radioactive dose in urine, feces, blood, and tissues after twice daily application of 50 µl of the 15%-formulation (SP106A) for five days. The systemic absorption of about 3-4% of the applied dose was seen in groups 4-6 after seven days of dermal dosing. It is unclear why such an increase in systemic absorption was noted between day 5 and 7 of dermal application. The sponsor proposes that the increased levels of absorption in these groups of rats may be attributed to loss of integrity of the skin at the site of application, formation of a depot of drug in skin following continued application (as indicated by increased radioactivity concentrations in skin at the site of application with an increase in the duration of treatment), or to suspected oral ingestion of the dermal doses.

Metabolism:

The sponsor states in the submission that BMS-203522 is not metabolized to any appreciable extent.

Plasma Protein Binding:

The sponsor states that BMS-203522 does not bind significantly to human plasma proteins based on the data reported in published literature.

Other Study:

Other Study #1:

Summary of the in vitro skin permeation profile of BMS-203522-1 (eflornithine) from different topical formulations during product development (CHOU-TW-99009)

Study Title:	Summary of the *in vitro* skin permeation profile of BMS-203522-1 (eflornithine) from different topical formulations during product development
Study No:	CHOU-TW-99009
Conducting laboratories:	Gillette Research Institute and
Date of study:	Final report dated May 1991 for Gillette Research Institute and May 1999 for
GLP compliance:	No

Within this summary, four *in vitro* skin permeation studies were performed by the Gillette Research Institute and

Study 1 compared the permeation of BMS-203522 in SP33 vehicle (25 mg formulation/1.2 cm^2 skin) at — and —— drug concentrations, using guinea pig and human

cadaver skin. Permeation across the guinea pig skin was ~2% of the applied dose for both the —— and —— BMS-203522 formulations (5.3 µg and 56.5 µg, respectively). Permeation across human cadaver skin was 0.44% (1.1 µg) and 0.27% (6.8 µg) of the applied dose for the — and — BMS-203522 formulations, respectively. Permeation of BMS-203522 through guinea pig skin was ~5-8 fold greater than in human cadaver skin.

Study 1 also evaluated the effect of permeation enhancers on the skin permeation profile of BMS-203522 in a ———— formulation, similar to SP33, using guinea pig skin. A total of six vehicles consisting of [ ] were evaluated using the guinea pig skin *in vitro* model. The results of this experiment demonstrated that the SP33 formulation was at least equal to or superior to the other formulations used in the study. Addition of a permeation enhancer and/or surfactant did not further enhance the permeation of BMS-203522 compared to the parent SP33 formulation.

Study 2 compared the permeation of BMS-203522 in the SP33 (— BMS-203522) and SP106 (SP106A-15% BMS-203522; SP106B- — BMS-203522; SP106C—— BMS-203522) formulations using guinea pig skin. The major objective of this study was to compare the *in vitro* permeation of BMS-203522 in the ———— SP33 formulation versus the ———— —— based SP106 formulation. A 10 mg dose of each respective [^{3}H] labeled BMS-203522 formulation was applied to the guinea pig skin and mounted on the modified —— diffusion chamber with a diffusional cross-section area of 1.8 cm^2 and normal saline solution containing 0.2% BSA as a receptor phase.

The absolute amounts of BMS-203522 that permeated in a 24 hour period were found to be nearly equal for the three SP106 formulations (range — µg). The amount of BMS-203522 absorbed from the SP33 formulation was ~47 µg. It was determined that this value was not significantly different from the amounts recovered from the SP106 formulations. The BMS-203522 permeation was about 3% and 5% of the applied dose for the SP106B and SP33 formulations, respectively. The BMS-203522 activity recovered in the skin samples was similar for the four formulations (range — µg BMS-2-3522). No significant differences were noted between the SP33 and SP106B formulations. Based on this result, the sponsor decided to evaluate the SP106 formulations further.

Study 3 evaluated the effect of change in manufacturing process and the effect of —— with —— component ———— on the permeation of BMS-203522 in the SP106 formulations. A 100 µl dose (~100 mg) of each formulation was applied to human cadaver skin mounted on modified —— diffusion chamber with a diffusional cross-section of 1.8 cm^2 with ———— as the receptor phase. The total absorption ranged from 1.03-1.34% for the different formulations tested at the end of the 24 hour treatment period. The results of this study indicated that no effect was noted on skin permeation of BMS-203522 from the SP106 vehicle with either a slight modification in the manufacturing process or incorporation of —— —— with a —— component ———— system.

Study 4 evaluated the effect of the manufacturing process on permeation of eflornithine in the to-be-marketed 15% BMS-203522 cream using human cadaver skin. [redacted] diffusion cells with a diffusional cross-section of 0.8 cm^2 were used in the study. A 5 $\mu l/cm^2$ dose of each respective formulation was applied to the human cadaver skin with distilled water as the receptor phase. Total absorption was found to be less than 0.2% of the applied dose at 48 hours for the three different formulations tested in this study. The results of the study determined that the BMS-203522 bioavailability was equivalent for the three different formulations. The most efficient manufacturing process, [redacted] was recommended for further evaluation in phase 3 clinical studies.

Summary of Pharmacokinetics:

The dermal absorption of [^{14}C]BMS-203522 in both mice and rats following single and multiple doses is quite low. This is consistent with the *in vitro* permeation results. The systemically absorbed doses of [^{14}C]BMS-203522 were primarily excreted unchanged in the urine. No apparent differences in the excretion, absorption and distribution of BMS-203522 were noted between males and female animals.

The sponsor has conducted human pharmacokinetic studies with the 15% BMS-203522 formulation (similar to SP106A). The sponsor states that the absorption, metabolism and excretion results of BMS-203522 from the animal studies were predictive of the results obtained in the human studies. Low systemic absorption of the dermal doses of BMS-203522 (~0.8% of the dose after 7 day dosing, 2X/day), the absence of metabolism of BMS-203522 *in vivo*, and the primarily renal excretion of BMS-203522 was noted in humans. The daily steady-state systemic exposure of hirsute women to BMS-203522, after twice-daily application of 0.5g of the 15% w/w cream formulation (150 mg BMS-203522/day) under conditions of clinical use, is estimated by the sponsor to be 185 ng·h/ml. The sponsor estimates that the steady-state plasma concentrations of BMS-203522 at 4 hr post-dose would be 8 ng/ml.

TOXICOLOGY:

Acute Toxicology Studies:

Acute Toxicology Study #1:

Acute dermal toxicity study in rabbits – limit test

Study Title:	Acute dermal toxicity study in rabbits – limit test
Study No:	GMEA 7597; 744-459
Amendment #, Vol #:	000, 12
Conducting laboratory:	[redacted]
Date of study initiation:	9/28/87
GLP compliance:	Yes

QA- Report: Yes (X) No ()
Methods:

The BMS-203522 (SP33) was administered topically to rabbits at a single dose of 5000 mg/kg (500 mg/kg BMS-203522). The trunk of each rabbit was wrapped with rubber damming and plastic collars were used to prevent possible ingestion of the test material. After the 24 hour exposure period, the collars and binders were removed and the exposure area was wiped with gauze moistened with water to remove any remaining material.

Dosing:

- *species/strain:* New Zealand White Rabbits
- *#/sex/group or time point:* 2/sex/dose
- *age:* 9-16 weeks
- *weight:* not stated
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* 5000 mg/kg SP33 (500 mg/kg BMS-203522)
- *route, form, volume, and infusion rate:* route = topical, 4.873 ml/kg

Drug, lot#, radiolabel, and % purity: Not stated

Formulation/vehicle: SP33 formulation, described in nonclinical formulation section previously

Observations and times:

- *Mortality:* twice daily for 14 days
- *Clinical signs:* daily for 14 days
- *Local dermal signs:* daily for 14 days
- *Body weights:* start of dosing, 48 hours post-dose, day 7 and at termination (day 14)
- *Gross pathology:* at sacrifice; 14 days after dosing

Results:

- **Mortality** No treatment related deaths were noted in this study.
- **Clinical signs** No treatment related clinical signs were noted in this study.
- **Local dermal signs** Dermal irritation consisting of very slight (grade 1) erythema with no edema was observed on the treated skin sites twenty-four hours following treatment. The irritation effects decreased after 72 hours post-treatment with very slight erythema persisting in one rabbit.
- **Body weights** No treatment related effects on body weight were noted in this study.

- **Gross pathology** No treatment related gross pathology effects were noted in this study.

Key Study Findings:

The acute lethal dermal dose of BMS-203522 cream (SP33 formulation) is greater that 500 mg/kg in rabbits. The — BMS-203522 cream (SP33 formulation) was characterized as slightly irritating in rabbits under the conditions of this study.

Acute Toxicology Study #2:

Acute oral toxicity study of SP106A in rats – limit test

Study Title:	Acute oral toxicity study of SP106A in rats – limit test
Study No:	GMEA 7765; —10403309
Amendment #, Vol #:	000, 12
Conducting laboratory:	
Date of study initiation:	5/15/91
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

An individual dose of the undiluted test material was administered via gavage to each rat.

Dosing:

- *species/strain:* Hsd:Sprague Dawley rats
- *#/sex/group or time point:* 5/sex/dose
- *age:* not stated
- *weight:* 192-206 grams
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* 10 g/kg SP106A (15% BMS-203522); (1500 mg/kg BMS-203522)
- *route, form, volume, and infusion rate:* route = oral (gavage), 9.90 ml/kg

Drug, lot#, radiolabel, and % purity: SP106A cream – Lot# 827/113B

Formulation/vehicle: SP106A formulation, described in nonclinical formulation section previously

Observations and times:

- *Mortality:* twice daily for 14 days
- *Clinical signs:* daily for 14 days

- *Body weights:* prior to dose administration and on day 14
- *Gross pathology:* at sacrifice; 14 days after dosing

Results:

- **Mortality** No treatment related deaths were noted in this study.
- **Clinical signs** One male rat exhibited soft stools on the day of dosing and one female rat exhibited soft stools the day after dosing. No other treatment related clinical signs were noted in this study.
- **Body weights** No treatment related effects on body weight were noted in this study.
- **Gross pathology** No treatment related gross pathology effects were noted in this study.

Key Study Findings:

The estimated oral LD_{50} for rats was determined to be greater than 10 mg/kg of SP106 cream (1500 mg/kg BMS-20355).

Special Toxicology Studies:

Special Toxicology Study #1:

Primary eye irritation study in rabbits

Study Title:	Primary eye irritation study in rabbits
Study No:	GMEA 7597; 744-456/744-457
Amendment #, Vol #:	000, 31
Conducting laboratory:	
Date of study initiation:	10/8/87
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

Ophthaine solution was instilled into each treated eye ~9-15 minutes prior to instillation of the test material. A 0.1 ml aliquot of the test material was placed into the conjuctival sac of the left eye of each rabbit with the right eye serving as the untreated control. The upper and lower lids were gently held together for 1 second to prevent loss of material. The treated eyes of 5 rabbits were not rinsed. The treated eyes of the second set of five rabbits were gently rinsed with 60 ml of warm tap water four seconds postinstillation.

Dosing:

- *species/strain:* New Zealand White rabbits
- *#/sex/group or time point:* Refer to dosing table below
- *age:* not stated
- *weight:* 2.36-2.81 kg
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* Refer to dosing table below
- *route, form, volume, and infusion rate:* route = topical (applied to the eye)

Dosing Table

Treatment	Dose (g/eye)	Number of Study Animals	
		Males	Females
SP33, Unwashed eye	0.1	3	2
SP33, Washed eye	0.1	2	3

Drug, lot#, radiolabel, and % purity: Not stated

Formulation/vehicle: Same as SP33 formulation, described in nonclinical formulation section previously

Observations and times:

- *Eye irritation:* Treated eyes were observed for ocular irritation at 1, 24, 48 and 72 hours and days 4 and 7 after treatment. At the 1 hour observation, sodium fluorescein was used in both eyes to aid in revealing possible corneal injury and at subsequent intervals, when necessary, to confirm any corneal damage. Irritation was graded and scored according to the Draize technique.

Results:

- **Eye irritation** SP33 was mildly irritating to the washed eyes in the rabbit. SP33 was moderately irritating to unwashed eyes of rabbits.

Key Study Findings:

SP33 (— BMS-203522 in ——— vehicle) was mildly irritating to the washed eyes and moderately irritating to unwashed eyes in rabbits under the conditions of this study.

Special Toxicology Study #2:

Primary eye irritation study of SP106A in rabbits – unwashed eye

Study Title: Primary eye irritation study of SP106A in rabbits – unwashed eye
Study No: GMEA 7765; — 10403310
Amendment #, Vol #: 000, 31
Conducting laboratory:
Date of study initiation: 5/14/91
GLP compliance: Yes
QA- Report: Yes (X) No ()
Methods:

Two drops of 0.5% proparacaine HCl, a preanesthetic, were placed on the corneal surface of the right eye of each rabbit ~5 minutes before test material instillation. Each rabbit received 0.1 ml of the test material placed into the everted lower lid of the right eye, with the left eye serving as the untreated control. The upper and lower lids were gently held together for 1 second to prevent loss of material. The eyes of the rabbits remained unwashed.

Dosing:

- *species/strain:* New Zealand White rabbits
- *#/sex/group or time point:* 2 males and 1 female
- *age:* not stated
- *weight:* 2.64-2.79 kg
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* 0.1 ml of SP106A
- *route, form, volume, and infusion rate:* route = topical (applied to the eye)

Drug, lot#, radiolabel, and % purity: SP106A – Lot# 827/133B

Formulation/vehicle: Same as SP106A formulation, described in nonclinical formulation section previously

Observations and times:

- *Eye irritation:* Treated eyes were observed for ocular irritation at 1, 24, 48 and 72 hours and days 4 and 7 after treatment. A sodium fluorescein examination was conducted at day 7. Irritation was graded and scored according to the Draize technique.

Results:

- **Eye irritation** SP106A produced slight to moderate conjunctival irritation in the unwashed eye of the rabbit. All treated eyes had returned to a normal

appearance by 72 hours after treatment. SP106A was minimally irritating to unwashed eyes of rabbits.

Key Study Findings:

SP106A (15% BMS-203522 in cream vehicle) was minimally irritating to unwashed eyes in rabbits under the conditions of this study.

Special Toxicology Study #3:

Primary eye irritation study of SP106A in rabbits – washed eye

Study Title:	Primary eye irritation study of SP106A in rabbits – washed eye
Study No:	GMEA 7765; 10403311
Amendment #, Vol #:	000, 31
Conducting laboratory:	[redacted]
Date of study initiation:	5/14/91
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

Two drops of 0.5% proparacaine HCl, a preanesthetic, were placed on the corneal surface of the right eye of each rabbit ~5 minutes before test material instillation. Each rabbit received 0.1 ml of the test material placed into the everted lower lid of the right eye, with the left eye serving as the untreated control. The upper and lower lids were gently held together for 1 second to prevent loss of material. The eyes of the rabbits were flushed with 60 ml of lukewarm tap water starting 4 seconds after test material instillation.

Dosing:

- *species/strain:* New Zealand White rabbits
- *#/sex/group or time point:* 2 males and 1 female
- *age:* not stated
- *weight:* 2.51-2.63 kg
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* 0.1 ml of SP106A
- *route, form, volume, and infusion rate:* route = topical (applied to the eye)

Drug, lot#, radiolabel, and % purity: SP106A – Lot# 827/133B

Formulation/vehicle: Same as SP106A formulation, described in nonclinical formulation section previously

Observations and times:

- *Eye irritation:* Treated eyes were observed for ocular irritation at 1, 24, 48 and 72 hours and days 4 and 7 after treatment. A sodium fluorescein examination was conducted at day 7. Irritation was graded and scored according to the Draize technique.

Results:

- **Eye irritation** SP106A produced slight conjunctival redness in the washed eye of two rabbits. All treated eyes had returned to a normal appearance by 72 hours after treatment. SP106A was minimally irritating to washed eyes of rabbits.

Key Study Findings:

SP106A (15% BMS-203522 in cream vehicle) was minimally irritating to washed eyes of rabbits under the conditions of this study.

Special Toxicology Study #4:

Guinea pig sensitization test (Buehler's technique modified)

Study Title:	Guinea pig sensitization test (Buehler's technique modified)
Study No:	GMEA 7597; 744-460
Amendment #, Vol #:	000, 31
Conducting laboratory:	
Date of study initiation:	10/26/87
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

For the induction phase, 0.5 ml of the test materials or 0.5 ml of a 0.05% solution of DNCB was used per application. The animals were dosed three times with seven days between each application. The animals were shaved ~24 hours before application. The test materials and the DNCB were applied on the left flank of the animals. Each solution was applied under a 1.5 x 2.0 inch adhesive dressing. The patches were secured in place with rubber damming and the animals were restrained for a six-hour contact period. After the contact period the restraining devices and patches were removed and any remaining compound was removed with lukewarm tap water. The treatment sites were graded for skin reactions ~24 hours after application according to the Draize method.

The challenge phase began thirteen days after the last induction phase application. The left flank and right flank of animals in the test and positive control groups and the left flank only of the negative control group were shaved at this point. The following day, 0.5 ml of the test materials and 0.5 ml of the 0.05% DNCB were applied to the animals under a 1.5 x 2.0 inch adhesive dressing. The patches were secured in the same fashion as in the induction phase and kept in place for six hours. On the day following removal of the restraining devices and patches, a depilatory cream was applied to the treatment sites. The cream was washed off ~30 minutes later. The treatment sites were graded for skin reactions at 24, 48 and 72 hours after removal of the patches according to the Draize method.

A second challenge dose was applied one to two weeks after the initial challenge. SP33 and DNCB were rechallenged on both the left and right flanks and the negative control run with SP33 was rechallenged on the left flank only. The negative control was defined as animals that received challenge with test material with no induction phase. No rechallenge phase was conducted for SP33V. A 0.5 ml aliquot of the test article or 0.5 ml aliquot of a 0.05% solution of the DNCB was applied under a 1.5 x 2 inch adhesive dressing. The patches were secured in the same fashion as in the induction phase and kept in place for six hours. On the day following the removal of the restraining devices and patches, the application sites were depilated as in the challenge phase and the treatment sites were graded for skin reactions at 24, 48, and 72 hours after removal of the patches according to the Draize method.

Dosing:

- *species/strain:* Hartley guinea pigs
- *#/sex/group or time point:* 10/sex in SP33 and SP33V groups, 5/sex in positive and negative control groups
- *age:* 4 weeks
- *weight:* 315-492 grams
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* 0.5 ml/application of SP33, SP33V or 0.05% DNCB
- *route, form, volume, and infusion rate:* route = topical, for additional information refer methods description above

Drug, lot#, radiolabel and % purity: SP33V – lot# NS-1922-98A
SP33 – lot# NS-1922-100B
1-chloro-2,4-dinitrobenzene (DNCB; positive control) – lot # B7C

Formulation/vehicle: Same as SP33 formulation, described in nonclinical formulation section previously

Observations and times:

- *Local dermal signs:* Treatment sites were graded for skin reactions according to the Draize method 24 hours after the induction phase treatment was complete. Treatment sites were graded for skin reactions according to the Draize method 24, 48 and 72 hours after both the challenge and rechallenge phase treatments were complete.

Results:

- **Local dermal signs** The positive control (0.05% DNCB) animals had no incidence of erythema during the induction phase of the study. During the challenge and re-challenge phases, the positive animals demonstrated an appropriate response.

  No incidence of erythema was noted in any of the SP33V or SP33 treated animals during the study in either the induction or challenge phases of the study or for SP33 treated animals during the rechallenge phase of the study.

Key Study Findings:

A positive response was noted in DNCB sensitized animals after elicitation with DNCB. No skin reaction was noted after challenge treatment with SP33V or SP33 or rechallenge with SP33. Therefore, SP33V and SP33 were considered to be non-sensitizers in guinea pigs under the conditions of this study.

Typically it is preferred to conduct the nonclinical sensitization test with the final to-be-marketed formulation. However, it is probably adequate to have conducted this test with the SP33 formulation compared with the to-be-marketed formula because the SP33 formulation contains additional inactive ingredients that may have a greater potential to induce a sensitization reaction. Since the SP33 formulation did not induce a nonclinical sensitization reaction, it is believed that the SP106 formulation probably would not elicit a nonclinical sensitization reaction. In addition, the medical officer (Denise Cook) has informed me that a clinical sensitization study has been conducted with the 15% BMS-203522 cream clinical formulation. The results of the clinical sensitization study were negative. Therefore, it will not be necessary for the sponsor to conduct another nonclinical sensitization study for this drug product.

Special Toxicology Study #5:

Phototoxicity study in Guinea pigs

Study Title: Phototoxicity study in Guinea pigs
Study No: 97687

Amendment #, Vol #: 000, 31
Conducting laboratory: Bristol-Myers Squibb Pharmaceutical Research Institute, Buffalo, New York
Date of study report: 9/5/97
GLP compliance: No
QA- Report: Yes () No (X)
Methods:

Four guinea pigs with depilated (Neet®) skin were temporarily restrained in stainless steel head yoke restraining boards. The skin on their backs was demarcated into four (3.15 x 3.5 cm) skin sites with adhesive tape. The 15% BMS-203522 lotion was applied at a dose of 0.1 ml/site to two diagonal skin sites on each of the four animals. Oxsoralen® 1% served as the positive control and was applied at a dose of 0.05 ml/site to the remaining two skin sites on each animal. The test materials were spread on the skin site using a gloved finger. Fifteen to twenty minutes after application of the test materials and prior to UVA exposure, the two sites on the right side of the first two animals were shielded from UVA exposure with cardboard. The eyes for all animals were also shielded from the light source with cardboard. The two sites on the left side of the first two animals and all four sites on the remaining animals were irradiated for 1 hour with UVA light (320-400 nm) emitted from six ——— blacklight bulbs, from a distance of ~12 inches. The total UVA dose administered was ~10 J/cm^2 cumulative irradiance measured with an ——— UVA Radiometer ———

Dosing:

- *species/strain:* male Hartley guinea pigs
- *#/sex/group or time point:* 4 males
- *age:* 6-8 weeks
- *weight:* 380-425 grams
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* 0.1 ml/application site of 15% BMS-203522, 0.05 ml/application site of 1.0% Oxsoralen (positive control)
- *route, form, volume, and infusion rate:* route = topical, for additional information refer to methods section above

Drug, lot#, radiolabel, and % purity: not stated

Formulation/vehicle: not stated

Observations and times:

- *Local dermal signs:* The treatment sites were graded for a phototoxic reaction (Draize erythema scale) at 24 and 48 hours following treatment and UVA exposure. If the mean of the erythema grades obtained for the UVA-exposed skin sites treated with the test material exceeds the mean of the unexposed sites (≥1), the test

material was considered phototoxic. The sponsor notes that the positive control, Oxsorlen®, is expected to produce photoxic reaction of ≥2.5.

Results:

- **Local dermal signs** No evidence of phototoxicity was observed on the skin sites treated with the 15% BMS-203522 lotion formulation at 24 and 48 hours following treatment and UVA exposure. All treated sites exhibited no erythema.

  On the sites irritated with UVA and treated with the positive control, 1% Oxsoralen®, moderate-severe to severe (grades 3 to 4) phototoxic reactions were recorded at the 24 and 48 hour grading intervals. The mean erythema grades at 24 and 48 hours were 3.8 and 4.0, respectively.

Key Study Findings:

The 15% BMS-203522 lotion formulation was not phototoxic to guinea pigs following UVA exposure under the conditions of this study.

Typically, this study would not be considered adequate since it was not conducted under GLP conditions. In addition, the exposure was only to UVA when it is preferred to have solar simulated exposure (UVA, UVB and visible). Apparently, an absorption spectrum was submitted to the IND with a chemistry information amendment dated 5/29/97. This absorption spectrum indicated that the drug product does absorb around — nm (UVB range). This absorption was attributable to the vehicle. Therefore, it is not surprising that the phototoxicity study conducted in guinea pigs with UVA exposure only is negative.

However, I have been informed by the medical officer, Denise Cook, that clinical phototoxicity and photoallergenicity studies have been conducted with the 15% BMS-203522 cream clinical formulation under conditions of adequate UVA and UVB exposure. The results of both the clinical phototoxicity and photoallergenicity studies were negative. Therefore, it will not be necessary for the sponsor to conduct another nonclinical phototoxicity study for this drug product.

Repeat Dose Dermal Irritation Studies:

Repeat Dose Dermal Irritation Study #1:

Repeat application dermal effect study in hamsters

Study Title: Repeat application dermal effect study in hamsters
Study No: — 744-573
Amendment #, Vol #: 000, 12
Conducting laboratory: [redacted]
Date of study initiation: 4/19/91
GLP compliance: Yes
QA- Report: Yes (X) No ()
Methods:

Each hamster was fitted with a plastic collar [redacted] three days prior to initiation of dosing. At day 1 (first day of dosing), an area on the left and right hindquarter region of ~2 x 2 inches was clipped closely with electric clippers. This area was then treated with a depilatory (Surex® Surgical Hair Remover Cream) for ~5 minutes, washed with tap water to remove the cream and dried with paper towel. Any hair regrowth was removed by clipping the site with scissors. The test material was applied using a microliter syringe and massaged into the skin using a gloved finger. Untreated animals did not receive any test material but were massaged as if being treated. Prior to each application the site was washed with water to remove any residual test material and dried with paper towel. The test materials were applied daily for thirteen days excluding weekends. Sites treated on Friday were not washed until the following Monday.

Dosing:

- *species/strain:* Lak:LVG(SYR) Golden Syrian hamsters
- *#/sex/group or time point:* Refer to dosing table below
- *age:* 69 days
- *weight:* 92-112 g
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* Refer to dosing table below
- *route, form, volume, and infusion rate:* route = topical, for additional information refer to table below

Dosing Table

Treatment	Dose Volume (ul/day)	Number of Male Study Animals
Untreated Control	0	6
SP106V (Vehicle Control)	10	6
SP106A (15% BMS-203522)	10	6
SP106B — BMS-203522)	10	6
SP106C — BMS-203522)	10	6

Drug, lot#, radiolabel, and % purity: SP106V – lot# 827/113BASE
SP106A – lot # 827/113B
SP106B – lot # 827/113A
SP106C – lot# 827/113C

Formulation/vehicle: Same as SP106 formulations, described in nonclinical formulation section previously

Observations and times:

- *Clinical signs:* twice daily
- *Local dermal signs:* daily
- *Body weights:* prior to first dose and at days 8 and 15
- *Gross pathology:* at sacrifice
- *Histopathology:* Animals were euthanized after 13 days of treatment. Samples of skin (one inch square) were taken from both the treated left flank (sham treated for untreated animals) and untreated right flank sites of each animal. Skin samples were fixed in 10% buffered formalin for histological analysis by hematoxylin and eosin staining.

Results:

- **Clinical signs** No treatment related deaths or clinical signs were noted in this study.
- **Local dermal signs** No treatment related local dermal signs were noted in this study.
- **Body weights** No treatment related effects on body weight were noted in this study.
- **Gross Pathology** No treatment related gross pathology effects were noted in this study.
- **Histopathology** No treatment related histopathology effects were noted in this study.

Key Study Findings:

BMS-203522 cream (SP106 formulation) at concentrations of ———15% was non-irritating in hamsters after repeat dose administration under the conditions of this study (13 days under occlusion, 10 µl of test article).

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Repeat Dose Dermal Irritation Study #2:

Repeat insult rabbit skin irritation study

Study Title:	Repeat insult rabbit skin irritation study
Study No:	GMEA 7597; 744-458
Amendment #, Vol #:	000, 12
Conducting laboratory:	[redacted]
Date of study initiation:	9/28/87
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

The liquid test materials were applied, 0.5 ml aliquots, directly to the skin under a 1 inch square gauze patch and secured in place with a [redacted] adhesive dressing. The trunk of each rabbit was wrapped with rubber damning. Plastic collars were used to prevent possible ingestion of the test material. Test material sites were rotated on each animal. After the 23 hour exposure period, the binders were removed and the exposure area was wiped with gauze moistened with water to remove any remaining test material. The application procedure was repeated for five consecutive days.

Dosing:

- *species/strain:* New Zealand White rabbits
- *#/sex/group or time point:* 2/sex total
- *age:* 9 – 18 weeks
- *weight:* not stated
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* 0.5 ml/day of either SP33 or SP33 vehicle
- *route, form, volume, and infusion rate:* route = topical

Drug, lot#, radiolabel, and % purity: not stated

Formulation/vehicle: Same as SP33 formulations, described in nonclinical formulation section previously

Observations and times:

- *Local dermal signs:* skin reactions were evaluated at 24 hours post-dose application (~1 hr after patch removal). Skin reactions were scored according to the Draize method.
- *Body weights:* at initiation of dosing and at study termination

Results:

- **Local dermal signs** The SP33 vehicle had PI index scores that ranged from 0.5 and 1.25 and was classified as mildly irritating. SP33 had PI index scores that ranged from 1.25 – 2.25 and was classified as mildly to moderately irritating.

- **Body weights** No treatment related effects on body weight were noted in this study.

Key Study Findings:

BMS-203522 cream (SP33 formulation; —; was mildly to moderately irritating in rabbits after repeat dose administration under the conditions of this study (5 days under occlusion).

Repeat Dose Dermal Irritation Study #3:

Repeat insult rabbit skin irritation study of samples SP106A, SP106B, SP106C and SP106V – (5 day repeat)

Study Title:	Repeat insult rabbit skin irritation study of samples SP106A, SP106B, SP106C and SP106V – (5 day repeat)
Study No:	GMEA 7765; — 10403312
Amendment #, Vol #:	000, 12
Conducting laboratory:	—
Date of study initiation:	5/13/91
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

Each test material (0.5 ml per site) was applied to the respective test sites. Each area of application was covered with a 1 inch x 1 inch gauze patch and secured with an adhesive dressing. The location of each test site was rotated from animal to animal to preclude a positional bias. The entire trunk of the animal was wrapped with a rubber dam binder. Collars were used to restrain the test animals during each 23 hour exposure period. After each 23 hour exposure period, the collars, binders, dressings and patches were removed from the animal. The test sites were wiped with gauze moistened with water to remove any remaining test material. The application procedure was repeated for 5 consecutive days.

Dosing:

- *species/strain:* New Zealand White rabbits
- *#/sex/group or time point:* 2/sex total
- *age:* not stated
- *weight:* 2.7 – 2.8 kg

- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* 0.5 ml/day of either SP106A, SP106B, SP106C or SP106V (vehicle)
- *route, form, volume, and infusion rate:* route = topical

Drug, lot#, radiolabel, and % purity: SP106V – lot# 827/113BASE
SP106A – lot # 827/113B
SP106B – lot # 827/113A
SP106C – lot# 827/113C

Formulation/vehicle: Same as SP106 formulations, described in nonclinical formulation section previously

Observations and times:

- *Local dermal signs:* The degree of erythema and edema was evaluated at 24 hours post-dose application (~1 hr after patch removal). Skin reactions were scored according to the Draize method.
- *Body weights:* at initiation of dosing and at study termination

Results:

- **Local dermal signs** SP106A had PI index scores that ranged from 0.75 – 3.75. SP106B had PI index scores that ranged from 1.00 – 3.50. SP106C had PI index scores that ranged from 1.00 – 2.75. SP106V had PI index scores that ranged from 1.00 – 3.00. All the test formulations were classified as mildly to moderately irritating.

- **Body weights** No treatment related effects on body weight were noted in this study.

Key Study Findings:

BMS-203522 cream (SP106 formulation) at concentrations of [redacted] 15% was mildly to moderately irritating in rabbits after repeat dose administration under the conditions of this study (5 days under occlusion).

Repeat Dose Dermal Irritation Study #4:

Repeat insult rabbit skin irritation study of samples SP106A, SP106B, SP106C and SP106V – (14 day repeat)

Study Title: Repeat insult rabbit skin irritation study of samples SP106A, SP106B, SP106C and SP106V – (14 day repeat)

Study No: GMEA 7768

Amendment #, Vol #: 000, 13
Conducting laboratory: [redacted]
Date of study initiation: 5/31/91
GLP compliance: Yes
QA- Report: Yes (X) No ()
Methods:

Each test material (0.5 ml per site) was applied to the respective test sites (~2.5 cm x 2.5 cm). The test materials were evenly distributed over each test site with a glass rod. The test areas were not covered, but were marked to denote the extent of test material exposure. The location of each test site was rotated from animal to animal to preclude a positional bias. Collars were used to restrain the test animals during each 23 hour exposure period. After each 23 hour exposure period, the test sites were wiped with gauze moistened with water to remove any remaining test material. The application procedure was repeated for 14 consecutive days.

Dosing:

- *species/strain:* New Zealand White rabbits
- *#/sex/group or time point:* 2/sex total
- *age:* not stated
- *weight:* 2.6 – 3.3 kg
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* 0.5 ml/day of either SP106A, SP106B, SP106C or SP106V (vehicle)
- *route, form, volume, and infusion rate:* route = topical

Drug, lot#, radiolabel, and % purity: SP106V – lot# 827/113BASE
SP106A – lot # 827/113B
SP106B – lot # 827/113A
SP106C – lot# 827/113C

Formulation/vehicle: Same as SP106 formulations, described in nonclinical formulation section previously

Observations and times:

- *Local dermal signs:* The degree of erythema and edema was evaluated at 24 hours post-dose application (~1 hr after patch removal). Skin reactions were scored according to the Draize method.
- *Body weights:* at initiation of dosing and at study termination

Results:

- **Local dermal signs** SP106A had PI index scores that ranged from 1.75 – 2.75. SP106B had PI index scores that ranged from 1.50 – 2.50. SP106C had PI

index scores that ranged from 1.50 – 2.75. SP106V had PI index scores that ranged from 1.75 – 3.25. All the test formulations were classified as mildly to moderately irritating.

- **Body weights** No treatment related effects on body weight were noted in this study.

Key Study Findings:

BMS-203522 cream (SP106 formulation) at concentrations of [redacted]15% was mildly to moderately irritating in rabbits after repeat dose administration under the conditions of this study (14 days; non-occlusion).

Repeat Dose Dermal Irritation Study #5:

14-day dermal irritation study in rabbits

Study Title:	14-day dermal irritation study in rabbits
Study No:	GMEA 7788
Amendment #, Vol #:	000, 13
Conducting laboratory:	[redacted]
Date of study initiation:	10/6/92
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

Approximately 24 hours prior to the initial application the fur was clipped from the treatment site (5 x 5 cm). The sites were re-clipped on an as needed basis. An aliquot of 250 µl was applied to the application site each day. This treatment was split so that 125 µl was applied in the morning and 125 µl was applied in the afternoon. The test material was rubbed into the skin with the aid of a finger cot. The treatment area was not occluded and the rabbits were not collared during the exposure. The treatment site was washed with water prior to each dose administration. The treatments continued for 14 days.

Dosing:

- *species/strain:* New Zealand White rabbits
- *#/sex/group or time point:* 2/sex/dose
- *age:* 13 weeks
- *weight:* 1.9 – 3.0 kg
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* 250 µl/day of either SP106A, SP106V, SP33 or SP33V
- *route, form, volume, and infusion rate:* route = topical

Drug, lot#, radiolabel, and % purity: SP106V – lot# 827/87
SP106A – lot # 827/88
SP33 – lot # 2167-39
SP33V – lot# 2167-37

Formulation/vehicle: Same as SP106 and SP33 formulations, described in nonclinical formulation section previously

Observations and times:

- *Mortality:* daily
- *Local dermal signs:* The degree of erythema and edema was evaluated daily, immediately before the first daily application of the test material (~1 hr after test article removal). Skin reactions were scored according to the Draize method.
- *Body weights:* at initiation of dosing and at study termination

Results:

- **Mortality** No treatment related effects on mortality were noted in this study.
- **Local dermal signs** SP106A had PI index scores that ranged from 0 – 0.25. SP106V had PI index scores that ranged from 0 – 0.375. SP33 had PI index scores that ranged from 0 – 0.125. SP106A, SP106V and SP33 were classified as minimally irritating. SP33V had PI index scores of 0. SP33V was classified as non- irritating.
- **Body weights** No treatment related effects on body weight were noted in this study.

Key Study Findings:

BMS-203522 cream [SP106A (15%) and SP33 — formulations] were minimally irritating in rabbits after repeat dose administration under the conditions of this study (14 days; non-occlusion).

Repeat Dose Dermal Irritation Study #6:

21-day repeat insult skin irritation study of SP106 formulations in rabbits

Study Title: 21-day repeat insult skin irritation study of SP106 formulations in rabbits
Study No: GMEA 7781
Amendment #, Vol #: 000, 13
Conducting laboratory: —

Date of study initiation: 5/28/92
GLP compliance: Yes
QA- Report: Yes (X) No ()
Methods:

Group 1 animals received one application/day of both test materials (24 hr application period). Group 2 animals received two applications/day of both test materials (~5 hours between the first and second applications). Each test material (80 µl) was applied to the respective test area (~4 x 4 cm) on each rabbit. The test materials were distributed over the test area by gently massaging into the skin with a gloved finger. The test area was marked to denote the extent of the test material exposure, left non-occluded and allowed to air dry. The location of each site of application was rotated on each animal to preclude a positional bias. After each 24 hour exposure period (Group 1) or before the first of the two daily applications (Group 2), the test materials were removed with paper towels moistened with water. The sites were allowed to air dry before being redosed. Collars were not used to restrain the test animals during each application period. The animals were treated in this manner for 21 consecutive days.

Dosing:

- *species/strain:* New Zealand White rabbits
- *#/sex/group or time point:* 2/sex/group
- *age:* not stated
- *weight:* 2.3 – 2.6 kg
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* 80 µl or 160 µl/day of either SP106A or SP106V (vehicle)
- *route, form, volume, and infusion rate:* route = topical

Drug, lot#, radiolabel, and % purity: SP106V – lot# 15302
SP106A – lot # 15303

Formulation/vehicle: Same as SP106 formulations, described in nonclinical formulation section previously

Observations and times:

- *Local dermal signs:* The degree of erythema and edema was evaluated according to the Draize technique just before the initial application on each treatment day. No observations for skin irriatition were done between the first and second daily test material applications for Group 2 animals.
- *Body weights:* at initiation of dosing and then weekly

Results:

- **Local dermal signs** SP106A applied once daily had a PI index score of 0.00 (non-irritating). SP106A applied twice daily had PI index scores that ranged from 0.25 – 0.50 (mildly irritating). SP106A applied once daily had PI index scores that ranged from 0.25 – 0.75 (mildly irritating). SP106V applied twice daily had a PI index score of 0.00 (non-irritating).

- **Body weights** No treatment related effects on body weight were noted in this study.

Key Study Findings:

BMS-203522 cream (SP106A formulation, 15%) ranged from non-irritating (once a day application) to mildly irritating (twice a day application) in rabbits after repeat dose administration under the conditions of this study (21 days; non-occlusion).

Repeat Dose Dermal Toxicology Studies:

Repeat Dose Toxicology Study #1:

14-day dermal exploratory toxicity study in hairless mice

Study Title:	14-day dermal exploratory toxicity study in hairless mice
Study No:	97659
Amendment #, Vol #:	000, 13
Conducting laboratory:	Bristol-Myers Squibb Pharmaceutical Research Institute, Buffalo, NY
Date of study report:	8/5/97
GLP compliance:	No
QA- Report:	Yes () No (X)

Objective:

The objective of this study was to explore the dermal tolerance of 15% BMS-203522 lotion (clinical formulation) in the hairless albino mouse. The sponsor stated that if 100 µl/mouse/day of the BMS-203522 formulation is tolerated in this study, then the same dose volume will be used as the top dose in a photocarcinogencity study. The sponsor notes that in the test formulation, 15% is the maximum concentration achievable with BMS-203522 and 100 µl/mouse is the maximum dose volume that can be practically administered to a mouse.

Methods:

Each test material formulation was administered for 14 consecutive days onto the skin of the back of each mouse at a dose volume of 100 µl/mouse, using a micropipette and spread over

a surface of ~10% (3x3 cm^2) using a gloved finger. The test and control formulations were applied undiluted to uncollared animals. The skin application site was the dorsal skin posterior to the interscapular region and extending posteriorly over the thoracic region to the lumbar area.

Dosing:

- *species/strain:* Hairless Abino Mice, SKHl (hr/hr)Crl
- *#/sex/group or time point:* 5/sex in treated group; 4/sex in control group
- *age:* 6-8 weeks
- *weight:* 23-26 gms
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* 100 µl/day of either 15% BMS-203522 lotion or vehicle lotion
- *route, form, volume, and infusion rate:* route = topical

Drug, lot#, radiolabel, and % purity: 15% BMS-203522 lotion – lot# 203522-M-03-B
Vehicle lotion – lot # 203522-M-06-A.

Formulation/vehicle: same as clinical formulation

Observations and times:

- *Clinical signs:* daily
- *Local dermal signs:* daily
- *Body weights:* prior to first dose and on days 3, 7, 10 and 14

Results:

- **Clinical signs** No treatment related deaths or clinical signs were noted in this study.
- **Local dermal signs** No treatment related effects on dermal irritation was noted in this study.
- **Body weights** No treatment related effects on body weight were noted in this study.

Key Study Findings:

No dermal effects were noted in this study after 14 repeat dose administration of 15% BMS-203522 lotion. It was determined that the maximum dose volume of 100 µl/mouse/day of the 15% BMS-203522 lotion should be well tolerated in a photocarcinogenicity study.