Repeat Dose Toxicology Study #2:

13-week dermal toxicology study in mice

Study Title: 13-week dermal toxicology study in mice
Study No: GMEA 7808
Amendment #, Vol #: 000, 13
Conducting laboratory: [redacted]
Date of study initiation: 5/6/94
GLP compliance: Yes
QA- Report: Yes (X) No ()
Methods:

An area of dorsal skin greater than 1 x 2 cm at the intrascapular region was clipped free of hair 1 day prior to treatment initiation and weekly thereafter. The corners of the application site (~1 x 2 cm) were marked within the shaved area with an indelible, nontoxic marking pen. The dosing material were applied once daily and rubbed into the skin with a glass rod. Tap water was used to dose the sham treated group and the treatment area was rubbed with a glass rod. Untreated animals were not rubbed with the glass rod. Approximately 6 hours after treatment the water sham, vehicle and test material sites were wiped with a wet (tap water) cotton ball. The application sites were not covered or occluded in this study. The test articles, vehicle or sham material was administered topically to the dorsal skin daily, 7 days per week, for 13 weeks.

Dosing:

- *species/strain:* Crl:CD-1 (ICR) albino mice
- *#/sex/group or time point:* Refer to dosing table below
- *age:* 6 weeks
- *weight:* 24.2-28.6 grams males; 19.5-23.0 grams females
- *satellite groups used for toxicokinetics or recovery:* NA
- *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 (ml/mouse/day)	Number of Study Animals	
		Males	Females
Untreated	0	8	8
Water Sham	100	8	8
SP106V	100	8	8
SP106A	50	8	8
SP106A	100	8	8

Drug, lot#, radiolabel, and % purity: SP106V – lot# 827/87
SP106A – lot # 827/88

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

Observations and times:

- *Clinical signs:* twice daily
- *Local dermal signs:* daily prior to next dose administration
- *Body weights:* weekly
- *Food consumption:* weekly
- *Gross pathology:* at sacrifice
- *Histopathology:* The following organs were preserved from each animal in 10% buffered formalin: bone marrow (femur), brain with stem, colon, cecum, rectum, duodenum, jejunum, ileum, heart, stomach, kidneys, lesions, liver with gallbladder, urinary bladder, mammary gland with skin, mesenteric lymph nodes and skin (treated and untreated).

  The kidneys, stomach, large and small intestines (colon, cecum, rectum, duodenum, jejunum, and ileum), liver, gallbladder, and treated and untreated skin sections from animals in the untreated and high dose SP106A groups were examined histologically. Lesions were examined histologically for all groups.

Results:

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

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- **Food Consumption** No treatment related effects on food consumption were noted in this study.
- **Gross pathology** No treatment related effects on gross pathology were noted in this study.
- **Histopathology** No treatment related histopathological effects were noted in this study.

Key Study Findings:

No treatment related toxicity was noted in this study. Therefore, the NOAEL for this study was 15% BMS-20355 cream (100 µl/day of SP106C formulation; 600 mg/kg/day or 1800 mg/m^2/day for a 25 g mouse) in the mouse. The NOAEL in this study is ~19 times the maximum human dose (1800 mg/m^2/day ÷ 92.5 mg/m^2/day).

Repeat Dose Toxicology Study #3:

26-week dermal toxicity study with SP106V, SP106A, SP33 and SP33V in rats

Study Title:	26-week dermal toxicity study with SP106V, SP106A, SP33 and SP33V in rats
Study No:	GMEA 7793; 744-576
Amendment #, Vol #:	000, 13-14
Conducting laboratory:	[redacted]
Date of study initiation:	11/15/92
GLP compliance:	Yes
QA- Report:	Yes (X) No ()
Methods:	

One day prior to the initial treatment, the hair on the dorsal scapular area of each rat was clipped with electric clippers and hair was clipped in the same area weekly thereafter. In order to minimize preening of the application site, the site was as far anterior as possible. The dose site area was marked at the corners with an indelible pen and remarked on an as needed basis. Test article was topically applied at a volume of 45 µl/application twice daily to a site ~9 cm^2 in the intrascapular region. The second application of the day was administered ~5 hours after the first. The test material was applied uniformly to the treatment site and gently rubbed into the skin using a gloved finger covered with a finger cot. Untreated animals were shaved and the skin was rubbed identically to treated animals. Application sites were not covered or occluded in this study. After the first day of treatment, each site was wiped with a paper towel dampened with water 60 minutes prior to the next first daily application. The treatment sites were not wiped prior to the second daily dose. The test articles, vehicle or sham material was administered topically to the dorsal skin daily, 7 days per week, for 26 weeks.

Dosing:

- *species/strain:* Crl:CD BR Sprague-Dawley rats
- *#/sex/group or time point:* Refer to dosing table below
- *age:* 8 weeks
- *weight:* 240-282 grams males; 177-215 grams females
- *satellite groups used for toxicokinetics or recovery:* Refer to dosing table below
- *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 Regimen	Dose Volume (µl/application)	Number of Study Animals	
			Males	Females
Untreated	None	0	10	10
SP106V	2X/day	45	10	10
SP106A	2X/day	45	10	10
SP33	2X/day	45	10	10
SP33V	2X/day	45	10	10

Drug, lot#, radiolabel, and % purity: SP106V – lot# 827/87
SP106A – lot # 827/88
SP33 – lot # NS2167-61
SP33V – lot# NS2167-59

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

Observations and times:

- *Clinical signs:* daily
- *Local dermal signs:* daily
- *Body weights:* weekly
- *Food consumption:* weekly
- *Hematology:* prior to terminal sacrifice (week 26)
- *Clinical chemistry:* prior to terminal sacrifice (week 26)
- *Gross pathology:* at sacrifice
- *Organs weighed:* adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes and thyroid/parathyroids
- *Histopathology:* The following organs were preserved from each animal in 10% buffered formalin: adrenals, aorta, bone marrow, brain, cecum, cervix, colon, duodenum, eyes, esophagus, epididymides, femur, heart, ileum, jejunum, kidneys, lesions, liver, lungs, lymph nodes (cervical, tracheobronchial and mesenteric), mammary

glands, mucles (thigh), ovaries, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerve, seminal vesicles, skin (treated and untreated), spinal cord, spleen, stomach, testes, thymus, thyroid (with parathyroids), trachea, urinary bladder, uterus and vagina.

Adrenals, bone marrow, brain, duodenum, femur, heart, ileum, jejunum, kidneys, lesions, liver, lung, mammary gland, ovaries, pituitary, spleen, stomach, testes, thyroid/parathyroids, urinary bladder, uterus and treated and untreated skin were examined microscopically for all animals.

- *Toxicokinetics:* Immediately prior to terminal sacrifice (~18 hours after the last dose during week 26) blood samples were obtained from each animal for analysis of plasma concentrations of BMS-203522. The plasma samples were analyzed using ——— method with ——— The lower limit of quantiation of this assay — µg/ml.

Results:

- **Clinical signs** No treatment related deaths or clinical signs were noted in this study.
- **Local dermal signs** Transient very slight erythema was noted during weeks 1, 2 and 3 in three SP106V male animals, three SP106A male animals and one SP106A female animal. No other treatment related local dermal effects were noted in this study.
- **Body weights** 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.
- **Hematology** No treatment related effects on hematology parameters were noted in this study.
- **Clinical chemistry** No treatment related effects on clinical chemistry parameters were noted in this study.
- **Organ weights** No treatment related effects on organ weights were noted in this study.

- **Gross pathology** No treatment related effects on gross pathology were noted in this study.
- **Histopathology** No treatment related histopathological effects were noted in this study.
- **Toxicokinetics** The plasma concentrations of BMS-203522 were below the lower limit of quantiation of ~~~ assay ~~µg/ml) in all animals.

Key Study Findings:

No treatment related toxic or irritant effects were noted in rats that were topically treated twice daily with SP106A or SP33 BMS-203522 formulations for 26 weeks under the conditions of this study. Therefore, the NOAEL in rats administered topical BMS-203522 cream daily for 26 weeks was 15% BMS 203522 cream (90 µl/day of SP106C formulation; ~68 mg/kg/day or ~400 mg/m^2/day for a 200 g rat) in this study. The NOAEL in this study is ~4 times the maximum human dose (400 mg/m^2/day ÷ 92.5 mg/m^2/day).

Repeat Dose Toxicology Study #4:

Dermal toxicology study with SP106V, SP106A and SP106C in rabbits

Study Title:	Dermal toxicology study with SP106V, SP106A and SP106C in rabbits
Study No:	GMEA 7783
Amendment #, Vol #:	000, 15
Conducting laboratory:	~~~
Date of study initiation:	8/3/92
GLP compliance:	No
QA- Report:	Yes () No (X)

Study Summary:

This dermal toxicity study was initiated to assess the toxicity of SP106V, SP106A and SP106C when applied to intact skin of rabbits twice daily, 7 days/week, for 26 weeks. The treatment site was not covered and not occluded in this study. Animals were assigned to treatment groups (10/sex/group) that received twice daily doses of SP106V, SP106A and SP106C applied to the dorsal dermal surface at doses of 125 µl/application (250 µl/day) over a 5 x 5 cm shaved area. An untreated control group was included in this study. Test article was rubbed into the test site with a finger cot and untreated control animals received similar extent of rubbing with a finger cot.

Signs of irritation became apparent in test article treated animals during the first week of treatment. Very slight to moderate to severe erythema, very slight edema, slight desquamation

and slight fissuring were seen at similar incidences for animals dosed with SP106V, SP106A or SP106C. This level of irritation was noted by day 15 of the study. No dermal irritation was noted for untreated control animals in this study. The study was terminated on day 15 due to the level of irritation observed in test article treated animals.

It is important to note that minimal dermal irritation was noted in a 14 day repeat dose dermal irritation study conducted in rabbits (2X/day treatment; 125 µl/application; 250 µl/day; with SP106A and SP33). In addition, a 21 day repeat dose dermal irritation study conducted in rabbits demonstrated mild dermal irritation (2X/day treatment; 80 µl/application; 160 µl/day; with SP106A). The contract lab's explanation for the degree of dermal irritation noted in this study was that different batches of rabbits may have a different sensitivity to irritation induced by the test article. Therefore, it was concluded that the rabbit may not be an appropriate model for evaluation of dermal toxicity due to the variability in dermal irritation responses with the specific test materials.

Key Study Findings:

The level of dermal irritation noted after dermal application of SP106V, SP106C and SP106A to rabbits was deemed unacceptable in this study. The study was terminated after 15 days of treatment. The sponsor decided that the rabbit was not an appropriate model to conduct dermal toxicity studies for the test article. It was decided to conduct the non-rodent long-term dermal toxicity in miniature swine. This study is described below.

It is interesting to note that the medical officer (Denise Cook) has informed that the clinical cumulative irritation study conducted under exaggerated use conditions did exhibit a positive irritant response for the 15% BMS-203522 cream clinical formulation. It would appear that the results of this study in rabbits provides support that the 15% BMS-203522 can elicit an irritant response.

Repeat Dose Toxicology Study #5:

15% BMS-203522 lotion: One year dermal toxicity study in miniature swine

Study Title:	15% BMS-203522 lotion: One year dermal toxicity study in miniature swine
Study No:	97631
Amendment #: Vol #:	000, 16
Conducting laboratory:	[redacted]
Date of study initiation:	8/19/97
GLP compliance:	Yes
QA- Report:	Yes (X) No ()

Methods:

The animals backs were clipped to remove hair one week prior to study start. The hair was clipped 24 hours prior to treatment initiation and then on an as needed basis. The treated area was located on the mid-thorax (~12 x 20 inches) and represented ~10% of the total body surface. The test material was dispensed using a syringe and spread onto the nonoccluded dose site using a gloved hand. Doses were administered twice daily to the clipped area. The first application was between 6:30 am to 8:30 am, then wiped four to five hours after application. The second application was between 11:30 am to 1:30 pm then wiped four to five hours after application. Any residual material was rinsed off with water and then wiped off the application site with a dry towel. The animals were treated with the test material topically twice each day for 52 consecutive weeks.

- *species/strain:* Hanford miniswine
- *#/sex/group or time point:* Refer to dosing table below
- *age:* 7 months
- *weight:* ~ 43 kg males; ~ 38 kg females
- *satellite groups used for toxicokinetics or recovery:* NA
- *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 (ml/kg/day)	Dose Level (mg/kg/day)	Number of Study Animals	
			Males	Females
Vehicle Lotion	1.0 (2 x 0.5)*	0	5	5
15% BMS-203522 lotion	0.1 (2 x 0.05)*	15	5	5
15% BMS-203522 lotion	1.0 (2 x 0.5)*	150	5	5

* - number in paratheses denotes the amount applied twice daily

Note: The contract lab stated that the highest dose was based on two factors. First, 15% was the highest concentration of the test material achievable with this formulation. Second, the 0.5 ml/kg aliquot was the maximum practical amount that could be applied to the back of the miniswine.

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

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

Observations and times:

- *Clinical signs:* daily
- *Local dermal signs:* weekly
- *Body weights:* weekly
- *Ophthalmoscopy:* prior to treatment and during week 26 and 52
- *Hematology:* prior to initiation of treatment and during weeks 13, 26, 39 and 52
- *Clinical chemistry:* prior to initiation of treatment and during weeks 13, 26, 39 and 52
- *Gross pathology:* at sacrifice
- *Organs weighed:* adrenal glands, brain, heart, kidneys, liver, spleen, testes and ovaries
- *Histopathology:* The following organs were preserved in 10% buffered formalin: adrenal glands, aorta, bone marrow (sternum), brain, cervix, epididymides, esophagus, eyes, femur, gallbladder, gross lesions, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, ileum, jejunum), kidneys, liver, lungs and bronchi, lymph nodes (mandibular, mesenteric), mammary gland, ovaries, pancreas, pituitary gland, prostate gland, salivary glands (mandibular), sciatic nerve, seminal vesicles, skeletal muscle, skin (treated and untreated), spinal cord, spleen, stomach, testes, thymus, thyroid gland, tongue, trachea, urinary bladder, uterus and vagina

  All tissues and organs were examined histologically for the control and high dose groups. Skin (treated and untreated) and all gross lesions were examined histologically for all animals.

Results:

- **Clinical signs** No treatment related effects on mortality or clinical signs were noted in this study.
- **Local dermal signs** No erythema or edema were noted in this study. No other local dermal effects were noted in this study.
- **Body weights** No treatment related effects on body weight were noted in this study.
- **Ophthalmoscopy** No treatment related ophthalmic examination findings were noted in this study.
- **Hematology** No treatment related hematologic findings were noted in this study.

- **Clinical chemistry** No treatment related clinical chemistry findings were noted in this study.
- **Organ weights** No treatment related effects on organ weights were noted in this study.
- **Gross pathology** No treatment related effects of gross pathology parameters were noted in this study.
- **Histopathology** No treatment related local (treated skin) or systemic microscopic changes in any of the tissues from the high dose animals (150 mg/kg BMS-203522) or in the treated skin from low dose animals (15 mg/kg BMS-203522) were noted in this study.

Key Study Findings:

No treatment related local dermal or systemic toxic effects were noted in minipigs that were topically treated twice daily with 15% BMS-203522 lotion (same as the clinical formulation) for 52 weeks under the conditions of this study. Therefore, the NOAEL in minipigs administered topical BMS-203522 lotion daily for 52 weeks was 15% BMS 203522 lotion (1 ml/kg/day; 150 mg/kg/day or 5250 mg/m^2/day) in this study. The systemic NOAEL in this study is ~57 times the maximum human dose (5250 mg/m^2/day ÷ 92.5 mg/m^2/day).

Reproductive Toxicology Studies:

Reproductive Toxicology Study #1:

15% BMS-203522 lotion: dermal study of fertility and early embryonic development in rats

Study Title:	15% BMS-203522 lotion: dermal study of fertility and early embryonic development in rats
Study No:	96676
Amendment #, Vol #:	000, 17
Conducting laboratory:	[redacted]
Drug lot# and batch#:	15% BMS-203522-01 lotion: Lot# 203522-M-03-A; Batch# IRB# B96B011 and FP# 96076 Vehicle lotion: Lot# 2303522-M-06-A; Batch# IBR# B96F006 and FP# 96140
Date of study initiation:	7/9/96
GLP compliance:	Yes
QA- Report:	Yes (X) No ()

Methods:

Species/strain:	Crl:CD®BR VAF Plus® Sprague Dawley rats
Age:	72 days
Weight:	326-365 g
#/sex/group:	25/sex/group
Route:	Topical, dermal
Doses:	0 (vehicle), 50, 150 and 450 mg/kg/day BMS-203522 lotion (0, 300, 900 and 2700 mg/m^2/day)
Dose Volume:	3, 0.33, 1 and 3 ml/kg/day (Note: Sponsor states that 3.0 ml\kg is the maximum dosage volume of the test article lotion formulation that can be practically administered to the application site and 15% is the maximum concentration of BMS-203522 attainable in the lotion formulation.)
Study Design:	Rats were acclimated to Elizabethan collars in the week prior to the start of treatment. Elizabethan collars were used during the treatment phase to prevent ingestion of test article. During the acclimation period, hair was shaved from the backs of the rats with electrical clippers (~10% of total body surface). Hair was shaved on an as needed basis throughout the study. The test article was applied to the shaved area and spread uniformly with a glass rod. The skin application site was covered with an adhesive bandage. The Elizabethan collar and bandage were removed after a 6 hour treatment period and the administration site was rinsed with a pad dampened with tap water and then dried with a second clean pad.
Treatment Schedule:	Males – 28 days prior to and though the cohabitation period Females – 15 days prior to and through the cohabitation period and to gestation day 7
Study Termination:	Males – sacrificed on study days 56-59 Females – sacrificed on gestational day 14

Observations:

Parental Animals

Mortality:	twice daily
Dermal Signs:	daily (Draize method)
Clinical Observations:	daily
Body Weights:	daily
Food Consumption:	weekly
Gross Pathology:	at sacrifice

Reproductive and Fertility Parameters:

Mating Performance:	daily during cohabitation

Sperm Evaluation:	cauda epididymal sperm count, viability and motility
Organ Weights:	Male – right testis, left testis, left epididymis, right epididymis, seminal vesicles and coagulating glands and prostate
Histopath analysis:	Males – testes, left and right epididymids, seminal vesicles and prostrate in control and high dose animals
Fertility parameters:	Number of corpora lutea, number and distribution of implantation sites, number of viable and nonviable embryos, litter size

Results:

Parental animals:

- **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.
- **Local dermal signs** — All groups, including vehicle, had signs of skin irritation (erythema, ulceration) at the application site. The incidence and severity of irritation exhibited a dose related trend.
- **Body weights** — 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.
- **Organ Weights** — No treatment related effects on organ weights were noted in this study.
- **Gross pathology** — No treatment related effects of gross pathology parameters were noted in this study.

Reproductive and Fertility Parameters:

- **Mating Performance** — No treatment related effects on mating performance in male or female animals were noted in this study.
- **Sperm Evaluation** — No biologically significant differences were noted in cauda epididymal sperm counts, sperm concentrations or sperm motility among the treatment groups.
- **Organ Weights** — The absolute and relative weights of epididymides, testes and male accessory sex glands were not significantly different among the four treatment groups.

- **Histopathology** No treatment related histopathological effects on the testes of the control and high dose animals were noted in this study.

- **Fertility Parameters** In females, estrous cycling (as evaluated by vaginal cytology) and mating and fertility parameters (number of estrous stages before or after treatment, days in cohabitation, fertility indices, and percentage pregnant rats/rats mated) were unaffected in this study. Mid-gestational fetal parameters were comparable among all four treatment groups (corpora lutea, implantations, litter size, viable and nonviable embryos and % postimplantation loss).

Summary and Evaluation:

No treatment related systemic toxicity or effects on reproductive or fertility indices were noted in rats that were topically treated with 15% BMS-203522 lotion (same as the clinical formulation) under the conditions of this study. Therefore, the NOAEL in rats for systemic maternal and reproductive toxicity is 450 mg/kg/day (2700 mg/m^2/day). The NOAEL in this study is ~29 times the maximum human dose (2700 mg/m^2/day ÷ 92.5 mg/m^2/day).

Reproductive Toxicology Study #2:

Developmental toxicity study in rats

Study Title:	Developmental toxicity study in rats
Study No:	744-578
Amendment #, Vol #:	000, 18
Conducting laboratory:	
Drug lot# and batch#:	SP106A: Lot# 882/060
Date of study initiation:	9/12/94
GLP compliance:	Yes
QA- Report:	Yes (X) No ()

Methods:

Species/strain:	Crl:CD®BR VAF Plus® Sprague Dawley pregnant female rats
Age:	9 weeks
Weight:	~250 grams
#/sex/group:	25/group; an extra 6 females were included in the high dose group for collection of plasma for drug level analysis only
Route:	Topical, dermal
Doses:	0 (tap water), 250 mg/kg and 450 mg/kg SP106A cream (0, 1500 and 2700 mg/m^2/day)
Dose Volume:	50 µl/cm^2, 25 µl/cm^2 and 50 µl/cm^2 (2x/day to 9 cm^2 area)

Study Design: An area of dorsal skin larger than the application site was shaved prior to treatment. Hair was shaved on an as needed basis throughout the study. The corners of the application site (~9 cm^2 area) were marked with a marking pen. The test article was applied twice daily (six hours apart). Each site was wiped with a wet (tap water) cotton ball prior to the first dose each day. The test article was applied to the treatment site with a glass rod. The application sites were not covered or occluded in this study.

Treatment Schedule: Gestation days 0 – 19
C-Section Schedule: Gestation day 20
Observations:

Parental Animals:

Mortality:	twice daily
Clinical Observations:	daily
Body Weights:	gestation days 0, 3, 7, 10, 14, 17 and 20
Food Consumption:	gestation days 0, 3, 7, 10, 14, 17 and 20
Toxicokinetics:	Day 19 – Plasma samples obtained from 6 high dose animals (3/6 sampled 4 hours after first daily application and 3/6 sampled 4 hours after second daily application). Day 20 – Plasma samples obtained from remaining high dose animals and 4 control animals just prior to sacrifice.
Gross Pathology:	at time of C-section a gross examination of abnormalities of the thoracic, abdominal and pelvic viscera was conducted for each rat. The uterus from each gravid female was excised, weighed, and examined for the number and placement of implantation sites, live and dead fetuses, early and late resorptions, and any abnormalities of the uterus or embryonic sac. The ovaries were examined for the number of corpora lutea)

Fetuses:

External Evaluation:	all fetuses were weighed, evaluated for gender and examined for gross external alterations.
Skeletal Malformations:	half of fetuses from each litter
Soft Tissue Malformations:	half of fetuses from each litter

Results:

Parental animals:

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

- **Clinical signs** Moderate erythema and slight epidermal scaling of the treatment site was noted in one high dose female and sores were noted in some low dose and high dose females. The contract lab reported this may have been due to shaving.

- **Body weights** Mean body weight values were significantly lower in low dose females at days 17 (↓6.7%) and 20 (↓12.8%) compared to control group values. Mean body weight values were significantly lower in high dose females at days 14 (↓4.6%), days 17 (↓10.2%) and 20 (↓19.3%) compared to control group values.

- **Food Consumption** Mean food consumption values were significantly lower in high dose females for the day 7-10 interval (↓6.9%), the day 14-17 interval (↓8.9%) and the day 17-20 interval (↓15.4%). No treatment related effect on food consumption was noted in low dose females.

- **Toxicokinetics** No drug was detectable in plasma from control animals. The lower limit of detection for the assay was — µg/ml. Plasma BMS-203522 levels (mean ± SEM) in high dose females are presented in the following table.

Time	BMS-203522 level (µg/ml)	Range (µg/ml)
4 hr post dose (day 19 am dose)	76.7 ± 26.2	29.4-120
4 hr post dose (day 19 pm dose)	26.2 ± 9.0	8.16-35.9
18 hr after last dose (day 20)	3.6 ± 0.77	0.67-19.6

- **Gross pathology**

The pregnancy rate was 100%. One high dose female had dark material around all of the placenta and no viable fetuses. Nine low dose dams and 21 high dose dams had no viable fetuses. Mean gravid uterine weights in the treated groups were lower than those of control animals due to the high incidence of in utero deaths. A significantly higher percentage of early resorptions and post implantation loss was noted in low (74.6%) and high dose females (94.6%) compared to control animals (6.7%), which resulted in a significantly lower percentage of mean live fetuses per litter. The mean percent of live fetuses was 93.3, 25.4 and 5.4 for control, low and high dose groups, respectively. The fetal sex ratio was similar for all dose groups. The mean covariate-adjusted fetal weights for all viable fetuses were significantly lower for low dose (↓25.7%) and high dose (↓32.6%) groups.

Fetuses:

Note: It is important to note that only 20 high dose fetuses were available for fetal evaluations compared to 356 control fetuses and 100 low dose fetuses.

- **External Malformations** No treatment related external malformations or variations were noted in this study.

- **Skeletal Malformations**

Two fetuses from the high dose group (both from the same litter) were noted to have skeletal malformations described as vertebral anomalies with or without associated rib anomalies (20% fetal incidence). Skeletal variations were noted in all groups but were significantly increased in treated groups. The contract lab notes that these skeletal malformations are considered to reflect delayed ossifications as a result of delayed in utero growth.

- **Soft Tissue Malformations**

No treatment related soft tissue malformations were noted in this study. Soft tissue variations noted included dilatation of lateral ventricles of the brain and increased renal pelvic cavitation. The contract lab notes that both variations are considered to reflect in-utero growth delays. The fetal incidence for dilatation of the lateral ventricles of the brain was the following: control (0%), low dose (25%) and high dose (80%). The fetal incidence for increased renal pelvic cavitation was the following: control (0%), low dose (5.9%) and high dose (10%).

Summary and Evaluation:

Body weight was significantly decreased in low and high dose dams and food consumption was significantly decreased in high dose dams. In utero growth retardation, as evidence by fetal weights and delayed ossification and development of the viscera was noted in both the low and high dose groups. Unfortunately neither a maternal or developmental NOAEL could be established in this study. Both the maternal and developmental NOAELs were less than 225 mg/kg/day based on the results of this study.

The sponsor attributes the results of this study to the fact that no precautions were taken to prevent access of the dams to the drug by covering the application sites or collaring the rats. Therefore, the sponsor proposed that it is likely that ingestion of the drug occurred during the study and the adverse maternal and developmental effects may have resulted from oral, and not dermal, exposure. The sponsor has repeated the dermal rat teratogenicity study with appropriate precautions. The results of this study are presented in the next study.

Reproductive Toxicology Study #3:

15% BMS-203522 lotion: dermal study of embryo-fetal development in rats

Study Title:	15% BMS-203522 lotion: dermal study of embryo-fetal development in rats
Study No:	96014
Amendment #, Vol #:	000, 19
Conducting laboratory:	Bristol-Myers Squibb , New Brunswick, NJ
Drug lot# and batch#:	15% BMS-203522-01 lotion: Lot# CN96005; Batch# IRB# B96B011 and FP# 96076 Vehicle lotion: Lot# 2303522-M-06-A; Batch# IBR# B96F006 and FP# 96140
Date of study initiation:	5/14/96
GLP compliance:	Yes
QA- Report:	Yes (X) No ()

Methods:

Species/strain:	Crl:CD®BR VAF Plus® Sprague Dawley pregnant female rats
Age:	12-13 weeks
Weight:	193-288 grams
#/sex/group:	20 for control group; 25/group for treatment groups; an extra 6 females were included in the high dose group for collection of plasma for drug level analysis only
Route:	Topical, dermal
Doses:	0 (vehicle control), 90 mg/kg, 250 mg/kg and 450 mg/kg BMS-203522 cream (0, 540, 1500 and 2700 mg/m^2/day)
Dose Volume:	50 µl/cm^2, 10 µl/cm^2, 25 µl/cm^2 and 50 µl/cm^2 (2x/day to 9 cm^2 area)
Study Design:	An area of dorsal skin larger than the application site was shaved prior to treatment. Hair was shaved on an as needed basis throughout the study. The corners of the application site (~9 cm^2 area) were marked with a marking pen. The test article was applied twice daily (six hours apart). Each site was wiped with a wet (tap water) cotton ball prior to the first dose each day. The test article was applied to the treatment site with a glass rod. The application sites were covered with an adhesive bandage and rats were collared (Elizabethan collar) to prevent ingestion of test article.
Treatment Schedule:	Gestation days 6 – 15
C-Section Schedule:	Gestation day 20

Observations:

Maternal Animals:

Mortality:	twice daily
Clinical Observations:	daily
Body Weights:	daily
Food Consumption:	daily
Toxicokinetics:	Day 15 – Plasma samples obtained from 6 high dose animals (3/6 sampled 4 hours after first daily application and 3/6 sampled 4 hours after second daily application). Day 20 – Plasma samples obtained from remaining six randomly selected pregnant high dose animals just prior to sacrifice.
Gross Pathology:	at time of C-section a gross examination of abnormalities of the thoracic, abdominal and pelvic viscera was conducted for each rat. The uterus from each gravid female was excised, weighed, and examined for the number and placement of implantation sites, live and dead fetuses, early and late resorptions, and any abnormalities of the uterus or embryonic sac. The ovaries were examined for the number of corpora lutea.

Fetuses:

External Analysis:	all fetuses were weighed, evaluated for gender and examined for gross external alterations.
Skeletal Malformations:	half of fetuses from each litter
Soft Tissue Malformations:	half of fetuses from each litter

Results:

Maternal animals:

- **Mortality** No treatment related effects on mortality were noted in this study.
- **Clinical signs** No treatment related clinical signs were noted in the control and low dose animals. Dose related increases in the incidence and severity of erythema at the application site was noted in dams in the mid and high dose groups. Slight to moderate erythema was noted in mid dose dams and slight to marked erythema was noted in high dose dams. 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.
- **Food Consumption** No treatment related effects on food consumption were noted in this study.

- **Toxicokinetics** Plasma BMS-203522 levels (mean ± SD) in high dose dams are presented in the following table. The lower limit of quantitation was —ng/ml.

Time	N	BMS-203522 level (µg/ml)
4 hr post dose (day 15 am dose)	3	2.34 ± 1.92
4 hr post dose (day 15 pm dose)	3	5.54 ± 3.81
at cesarean section (day 20)	6	0.014 ± 0.008

- **Gross pathology** No treatment related gross lesions were identified at the maternal necropsy. No treatment related changes in any maternal or litter parameters were noted at the time of ceasarean sectioning.

Fetuses:

- **External Malformations** No treatment related external malformations or variations were noted in this study.
- **Skeletal Malformations** No treatment related skeletal malformations or variations were noted in this study.
- **Soft Tissue Malformations** No treatment related soft tissue malformations or variations were noted in this study.

Summary and Evaluation:

Precautions were taken to prevent ingestion of BMS-203522 by dams in this study. Levels of BMS-203522 in maternal plasma in this study compared to the previous study show a reduced extent of absorption of the test article after dermal application in this study. The circulating plasma levels of BMS-203522 were 11 to 14 fold higher in the previous study compared with this study. The presence of a dose dependent erythema in this study, which was not seen in the previous study, may have been related to the use of occlusion in this study.

The results of this study indicate that topically applied 15% BMS 203522 lotion is not teratogenic or embryolethal to rats under the conditions of this study. The maternal NOAEL was this study was 90 mg/kg/day (540 mg/m^2/day; 10 µl/cm^2) under the conditions of this study. The fetal NOAEL was 450 mg/kg/day (2700 mg/m^2/day; 50 µl/cm^2) under the conditions of this study.

It is important to note that there were still µg/ml levels of BMS-203522 measured in this dermal study even when precautions for oral ingestion were made. It is also important to note that dams were dosed from days 0-19 in the previous study and on days 6-15 in this study. Even if the test article had not been ingested in the first study, the additional duration of exposure and

potential earlier exposure of the fetus might have contributed to the increased incidence of fetal loss noted in the previous study. The level of early absorptions accounted for the increased incidence of fetal loss noted in the previous study.

Reproductive Toxicology Study #4:

15% BMS-2-3522 lotion: dermal study of embryo-fetal development in rabbits

Study Title:	15% BMS-2-3522 lotion: dermal study of embryo-fetal development in rabbits.
Study No:	96677
Amendment #, Vol #:	000, 20
Conducting laboratory:	
Drug lot# and batch#:	15% BMS-203522 lotion: Lot# 203522-M-03-A; Batch# IRB# B96B011 and FP# 96076 Vehicle lotion: Lot# 2303522-M-06-A; Batch# IBR# B96F006 and FP# 96140
Date of study initiation:	8/14/96
GLP compliance:	Yes
QA- Report:	Yes (X) No ()

Methods:

Species/strain:	New Zealand White pregnant female rabbits
Age:	5-5.5 months
Weight:	2.80-4.02 kg
#/sex/group:	20/group
Route:	Topical, dermal
Doses:	0 (vehicle control), 30 mg/kg, 90 mg/kg and 300 mg/kg BMS-203522 cream (0, 330, 990 and 3300 mg/m^2/day)
Dose Volume:	2, 0.2, 0.6 and 2 ml/kg/day (The sponsor notes that a dose volume of 2.0 ml/kg/day is the highest volume that can be practically administered to rabbits.)
Study Design:	The backs of rabbits were shaved prior to treatment (treatment area = 10 x 20 cm; ~10% total body surface area). Hair was shaved on an as needed basis throughout the study. Each rabbit was fitted with an Elizabethan collar to prevent ingestion of the test article. The required dosage volume was dispensed to the treatment area and spread with a glass rod. The skin application site was covered with non-adherent gauze pads and the pads were wrapped with Micropore tape. The administration site was rinsed with a tap water dampened pad and dried

with a second pad 6 hours after treatment. Frequency of treatment was once daily.

Treatment Schedule: Gestation days 6 – 18
C-Section Schedule: Gestation day 29
Observations:

Maternal Animals:

Mortality:	twice daily
Clinical Observations:	daily
Body Weights:	daily
Food Consumption:	daily
Gross Pathology:	at time of C-section a gross examination of abnormalities of the thoracic, abdominal and pelvic viscera was conducted for each rat. The uterus from each gravid female was excised, weighed, and examined for the number and placement of implantation sites, live and dead fetuses, early and late resorptions, and any abnormalities of the uterus or embryonic sac. Placentae were evaluated for abnormalites in size, shape and color. The ovaries were examined for the number of corpora lutea.

Fetuses:

External Analysis:	all fetuses were weighed, evaluated for gender and examined for gross external alterations.
Skeletal Malformations:	all fetuses
Soft Tissue Malformations:	all fetuses

Results:

Maternal animals:

Note: The sponsor states that there was difficulty in removing all of the test material for the application site on the rabbits prior to reapplication of the test material in this study. In addition, it is noted in the final study report that rabbits in each group chewed, partially removed or removed the bandage on one or more days during the exposure period. It was noted that one or two rabbits in each group were able to remove the Elizabethan collar once during the dosage period. It was felt that the rabbits were probably trying to reduce the irritation produced by the test article. This result would indicate that the rabbits had some potential for oral ingestion of the test article.

- **Mortality** One high dose rabbit was found dead on gestational day 28.

- **Clinical signs** Skin irritation was present in all groups, including control. Signs included erythema, edema, flaking, scabbing, fissuring and ulceration at the application sites. The incidence and severity were related to dose and to duration of treatment. Skin lesions were severe enough in two high dose animals that dosing was discontinued after gestational day 16. Both of these animals subsequently aborted their litters. A total of four rabbits from the high dose group aborted on gestational days 10 (1), 21 (2) or 23 (1).

  Four high dose rabbits had a red substance found in the cage pan that was associated with resorption or abortion of litters. An increased incidence of vocalization and of soft or liquid feces was noted in high dose rabbits. In the high dose rabbit that died, marked body weight loss and reduced feed consumption were noted after gestational day 21. Also, dehydration, perianal fecal matter, white perinasal and periocular substances, absence of feces and emaciation were noted in this high dose rabbit.

- **Body weights** A significant decrease in body weight (↓6.9% by gestational day 29) was noted in high dose animals compared to control animals.

- **Food Consumption** A significant decrease in food consumption (↓8.7% over the period from gestational day 6 – 29) was noted in high dose animals compared to control animals.

- **Gross pathology** Increased fetal resorptions, increased post-implantation losses, decreased live litter size and reduced fetal body weights were noted in the high dose group. No treatment related effects on the average number of corpoa lutea, implantations or percent live male fetuses (sex ratio) were noted in this study.

  Dry white contents were observed in the left horn of the uterus in the high dose animal that died in this study. No other treatment related gross lesions were noted in this study.

Fetuses:

- **External Malformations** No treatment related external malformations or variations were noted in this study.

- **Skeletal Malformations** No treatment related skeletal malformations or variations were noted in this study. Skeletal alterations that were significantly different from control in the high dose group (hole in parietal bones, sternal centra fused) were within the range of historical controls.

- **Soft Tissue Malformations** No treatment related soft tissue malformations or variations were noted in this study.

Summary and Evaluation:

It would appear that an increase in systemic exposure may have occurred by either some oral ingestion of the test article or increased dermal absorption through severely irritated skin. Unfortunately, no pharmacokinetic data was obtained in this study. Therefore, it is difficult to definitely state that this was the case. However, based on the extent of maternal toxicity apparent in the high dose group, it is anticipated that significant systemic exposure occurred in the high dose animals in this study.

Maternal signs of toxicity included local skin irritation in vehicle and treatment groups. The skin irritation was severe enough in the high dose group to warrant discontinuation of treatment in two animals and to result in abortions in four animals. One death occurred in the high dose group, which may be attributable to treatment. A significant degree of fetal lethality, which may be related to increased systemic exposure through severely damaged skin at the application site or to some degree of oral ingestion, was noted in the high dose group.

Topically applied BSM 203522 lotion did not exhibit any teratogenic potential in rabbits under the conditions of this study. The maternal and developmental NOAELs were 90 mg/kg/day (990 mg/m^2/day, 0.6 ml/kg) under the conditions of this study.

Reproductive Toxicology Study #5:

Perinatal and postnatal study with eflornithine hydrochloride in rats

Study Title:	Perinatal and postnatal study with eflornithine hydrochloride in rats
Study No:	T-86-20
Amendment #, Vol #:	000, 21
Conducting laboratory:	[redacted]
Drug lot# and batch#:	Eflornithine hydrochloride: Lot# — 71,782A-131
Date of study initiation:	4/9/86
GLP compliance:	Yes
QA- Report:	Yes (X) No ()

Methods:

Species/strain:	Sprague-Dawley pregnant female rabbits
Age:	not stated
Weight:	231 – 308 grams
#/sex/group:	20/group
Route:	Oral, Drinking water

Doses: [redacted] BMS-203522 in drinking water
(0, 223, 625 and 1698 mg/kg/day)
(0, 1338, 3750 and 10188 mg/m^2/day)

Treatment Schedule: Gestation day 15 – lactation day 22
Observations:

F_0 Maternal Animals:

Clinical Observations:	daily
Body Weights:	weekly
Food Consumption:	weekly
Water Consumption:	every 2 or 3 days
Gross Pathology:	A gross necropsy evaluation of dams was conducted after the litters were weaned. Uteri were examined for implantation sites at the time of the gross necropsy.

F_1 Litters:

Clinical Observations:	daily (recorded number of live, dead and cannibalized pups)
Pup Weights and Sex:	days 1, 5, 15 and 22 after parturition
Litter Reduction:	day 5; litters reduced 4 males and 4 females
Developmental and Behavioral Tests:	performed during the nursing period; surface righting day 5, auditory startle ([redacted] whistle) day 15, examination for eye opening day 16 and pupil contraction day 20
Sexual maturation:	performed after weaning (day 22); 1 male and 1 female from each litter were raised to sexual maturity for mating

F_1 Paternal Animals:

Note: All pups (F_1) that were retained at weaning were maintained in their respective dose groups on deionized water to sexual maturity. At approximately 95 days of age each female rat was cohabitated with a male rat (non-sibling) of the same dose group until evidence of mating was noted or for 2 weeks.

Clinical Observations:	daily
Body Weight:	weekly
Food Consumption:	weekly
Gross Pathology:	Pregnant female rats were sacrificed on gestational day 16 and given a gross necropsy examination. The number of live, dead, resorbed fetuses and corpora leutea were recorded for each pregnant female animal. Male rats were sacrificed immediately after the female rats and given a gross necropsy examination.

Results:

F_0 Maternal animals:

- **Clinical signs** — No treatment related maternal clinical observations were noted in this study.
- **Body weights** — Maternal body weight was reduced during the treatment period in the mid (↓3.3%) and high (↓6.7%) dose groups compared to control animals.
- **Food Consumption** — Maternal food consumption was reduced during the treatment period in mid (↓9.1%) and high (↓20.0%) dose groups compared to control animals.
- **Water Consumption** — Maternal water consumption was reduced during the treatment period in the high (↓30.8%) dose group compared to control animals.
- **Gross pathology** — No treatment related effects on gross pathology parameters were noted in male or female animals.

No treatment related effects on length of gestation, number of implants, number of live and dead pups and sex ratio were noted in this study.

F_1 Litters:

- **Clinical signs** — Survival rate of the high dose group was decreased on day 5 due to the loss of 3 litters shortly after birth due to cannibalism.
- **Pup weights** — Pup weights were significantly reduced in the mid and high dose groups on days 5, 15 and 22 (↓8.1% and 23.0% on day 22, respectively) compared to control animals.
- **Behavioral Tests** — No significant treatment related effects on surface righting, auditory startle, eye opening and pupillary reflex were noted in this study. A slight difference was noted in high dose pups in all parameters. The contract lab noted that this may be due the reduced growth and development (weight) of the pups in the high dose group. This is an acceptable explanation.

F_1 Paternal Animals:

- **Clinical signs** — No treatment related clinical observations were noted in F_1 paternal animals in this study.

- **Body weights** Mean body weights of the male rats in the high dose group remained significantly less than control during the first 5 weeks after weaning. Mean body weights of female rats in the mid and high dose groups remained significantly less than control animals during most of the growth and reproduction period.

- **Food Consumption** No treatment related effects on food consumption were noted in F_1 parental animals in this study.

- **Fertility Index** The fertility index of the high dose group was significantly lower than control animals (76.5% and 89.5%, respectively).

- **Gross Pathology** No treatment related effects on gross pathology parameters were noted in male or female F_1 parental animals.

  No treatment differences in the number of corpora lutea, implants, viable and dead fetuses and resorptions were noted in F_1 maternal animals.

Summary and Evaluation:

Eflornithine hydrochloride (BMS 203522) produced mild toxicity signs of decreased maternal and pup body weights in the mid and high dose groups under the conditions of this study. Eflornithine hydrochloride caused changes in the F_1 generation offspring at doses that also caused mild maternal toxicity. The systemic maternal, neonatal and developmental NOAEL was — Eflornithine hydrochloride (223 mg/kg/day; 1338 mg/m^2/day) under the conditions of this study.

Labeling Recommendations:

It is recommended that Pregnancy category C is the appropriate pregnancy category for the 15% BMS 203522-lotion. This recommendation is based on the results of the dermal teratogenicity studies conducted in rats and rabbits and the low level of systemic absorption (~0.80%) noted after 7 day repeat administration of the maximum dose of the 15% BMS 203522 lotion (twice/day) in humans. ✓

Genetic Toxicology Studies:

Genetic Toxicology Study #1:

BMS 203522 ames reverse-mutation study in Salmonella and Escherichia Coli

Study Title: BMS 203522 ames reverse-mutation study in *Salmonella* and *Escherichia Coli*

Study No: 97647

Study Type: Ames test
Study Endpoint: *In vitro* bacterial mutagenicity
Amendment #, Vol #: 000, 21
Conducting Laboratory: Bristol Myers Squibb Pharmaceutical Research Institute, Syracuse, NY
Study Dates: report date 7/17/97
GLP Compliance: Yes
QA- Reports: Yes (X) No ( )

Methods:

Indicator Strains: *Salmonella typhimurium* strains: TA98, TA100, TA1535 and TA1537
E. coli strains: WP2 uvrA
Test Article: BMS 203522
Drug Lot Number: BMS-203522-01, batch# 28
Vehicle Control: Distilled water
Concentrations: 312.5, 625, 1250, 2500 and 5000 µg/plate (three replicate cultures/concentration; ± metabolic activation)
Cytotoxic Effects: No cytotoxicity noted at highest dose (highest dose meets ICH requirements)
Criteria Evaluated: Reversion frequency, viability, integrity of background lawn
Metabolic Activation System: Aroclor 1254-induced rat liver S9

Positive Controls, Negative Controls, and Concentrations Tested

Strain	Mutagen (Positive Control)	Solvent (Negative Control)	Concentration (µg/plate)
With Activation			
TA98	2-Aminoanthracene	DMSO	2.5
TA100	2-Aminoanthracene	DMSO	2.5
TA1535	2-Aminoanthracene	DMSO	2.5
TA1537	2-Aminoanthracene	DMSO	2.5
WP2 uvrA	2-Aminoanthracene	DMSO	10
Without Activation			
TA98	2-Nitrofluorene	DMSO	2
TA100	Sodium Azide	Distilled water	2
TA1535	Sodium Azide	Distilled water	2
TA1537	9-Aminoacridine	DMSO	100
WP2 uvrA	Methyl Methane-Sulfonate	N/A	2.5 µl/plate

Results:

No evidence of cytotoxicity was noted at the highest concentration tested in this assay. The histidine+ and tryptohan+ revertant values were not significantly higher in BMS 203522 treated cultures than in negative controls. Positive controls yielded an appropriate increase in revertants in each tester strain. An independent confirmatory assay yielded similar results.

Summary:

BMS 203522 was negative in the Ames test under the conditions of this assay.

Genetic Toxicology Study #2:

BMS 203522 cytogenetics study in primary human lymphocytes

Study Title:	BMS 203522 cytogenetics study in primary human lymphocytes
Study No:	97651
Study Type:	Chromosome Aberration Assay
Study Endpoint:	*In vitro* mammalian cell chromosomal aberration
Amendment #, Vol #:	000, 21
Conducting Laboratory:	Bristol Myers Squibb Pharmaceutical Research Institute, Syracuse, NY
Study Dates:	report date 8/25/97
GLP Compliance:	Yes
QA- Reports:	Yes (X) No ( )

Methods:

Indicator Strains:	Human Lymphocytes
Test Article:	BMS 203522
Concentrations:	300, 600, 1200 and 2400 (~10 mM; upper limit ICH dose) µg/ml (four replicate cultures/concentration; 24 hour exposure without metabolic activation; 5 hour exposure with metabolic activation)
Drug Lot Number:	BMS-203522-01, batch# 28
Vehicle Control:	Distilled water
Positive Controls:	Mitomycin C (0.1 µg/ml, non-activated system) Cyclophosphamide (4 µg/ml, activated system)
Cytotoxic Effects:	No cytotoxicity noted at highest dose in the range finding study (highest dose meets ICH requirements)
Criteria Evaluated:	Chromosome aberrations
Metabolic Activation System:	Aroclor 1254-induced rat liver S9

Results:

No increase in chromosomal aberrations as a result of exposure to BMS-203522 was noted in this study. Mitotic indices were decreased by up to 28% as compared to negative controls in exposures without metabolic activation. This was considered to be evidence of minimal cytotoxicity. Positive controls yielded an appropriate increase in chromosomal aberrations.

Summary:

BMS 203522 was negative in the human lymphocyte chromosomal aberration assay under the conditions of this assay.

Genetic Toxicology Study #3:

15% BMS 203522 lotion: dermal micronucleus study in rats

Study Title:	15% BMS 203522 lotion: dermal micronucleus study in rats
Study No:	97654
Study Type:	Rat Micronucleus Assay
Study Endpoint:	*In vivo* bone marrow micronucleus formation
Amendment #, Vol #:	000, 21
Conducting Laboratory:	Bristol Myers Squibb Pharmaceutical Research Institute, Syracuse, NY
Study Dates:	report date 9/5/97
GLP Compliance:	Yes
QA- Reports:	Yes (X) No ( )

Methods:

Species/Strain:	Sprague-Dawley rats
#/Sex/Group:	5/sex/group
Test Article:	15% BMS 203522 lotion
Dose:	0, 100, 300 and 900 mg/kg (Note: the high dose represents the highest dose volume that could be applied dermally to the rat and the maximum achievable concentration of the drug in this vehicle.)
Dose Volume:	6, 0.67, 2.0 and 6.0 ml/kg
Drug Lot Number:	BMS-203522-01, batch# B97A004
Vehicle Control:	Vehicle lotion
Positive Control:	Cyclophosphamide (dose = 7 mg/kg; dose volume = 10 ml/kg)
Route:	Dermal for the 15% BMS 203522 lotion (applied to ~10% of total body surface area; animals wore elizabethan collars during drug application period to prevent ingestion of test article; collars were removed after 6 hours and any remaining test article was wiped off) Intraperitoneal for cyclophosphamide

Duration: 3 days
Criteria Evaluated: Micronuclei in polychromatic erythrocytes

Results:

No treatment related deaths, signs of systemic toxicity, signs of bone marrow toxicity (as evaluated by decreases in polychromatic erythrocytes) were noted in this study. No change in frequency of micronucleated polychromatic erythrocytes were noted in test article treated groups with the exception of the mid-dose males. Males in the mid dose group exhibited a significant increase in micronucleated polychromatic erythrocytes (0.29% vs 0.17% for controls). However, the value for mid-dose males was within the range for historical controls and is not biologically significant. Positive controls yielded an appropriate increase in micronuclei in polychromatic erythrocytes.

Summary:

BMS 203522 was negative in the dermal mouse micronucleus assay under the conditions of this assay.

Reviewer's comment: Test article administration was by topical application rather than oral or parental administration. Positive results may have been masked by limited transdermal absorption in rats. A better study design would have been to administer the drug substance systemically in this experiment and justify the relevance (or lack of) of any positive results in human topical dosing by demonstration of the degree of transdermal absorption in human subjects.

The high dose (900 mg/kg) is equivalent to 5400 mg/m^2. The maximum daily human dose was estimated at 2.5 mg/kg (92.5 mg/m^2). The high dose of 15% BMS 203522 lotion tested in the dermal rat micronucleus assay is ~58 fold greater than the maximum daily human dose. This is an acceptable safety margin for the dermal rat micronucleus assay.

Overall Genetic Toxicology Summary:

BMS 203522 was not genotoxic in the ICH standard battery of genotoxicity assays (Ames test, chromosomal aberration assay and dermal rat micronucleus assay).

The rat micronucleus test was conducted by the dermal route for the test article. It would have been advisable to have conducted the rat micronucleus assay via the systemic route to increase the systemic exposure to the test article in the rat micronucleus assay. However, the high dose of 15% BMS 203522 lotion tested in the dermal rat micronucleus assay is ~58 fold greater than the maximum daily human dose. This is an acceptable safety margin for the dermal rat micronucleus assay.

Carcinogenicity Studies:

Carcinogenicity Study #1:

Twelve-month photocarcinogenicity study in hairless mice

Study Title:	Twelve-month photocarcinogenicity study in hairless mice
Study Number:	97719
Volume Numbers:	22, 23
Test Facility:	[redacted]
Study Date(s):	9-17-97 to 9-13-98
Date of Submission:	9-27-99
GLP Compliance:	Yes
QA- Report:	Yes (X) No ()
Study Type:	One year photo co-carcinogenicity study in hairless mice
Species/strain:	Crl:SKH1-hr BR (albino hairless) mice
#/sex/ group:	36/sex/group
Age at start of study:	49 days old (males: 26 - 36 g, females: 20 - 31 g)
Animal housing:	The mice were individually housed in stainless steel cages with floor area of at least 96.8 cm^2 and a height of at least 12.7 cm.
Drug Lot/Batch number(s):	Vehicle lotion (203522-M-06-A) – lot# B97A003 15% BMS 203522 lotion (203522-M-03-B) – lot# B97A004
Drug Purity:	Conducted by sponsor, no impurities were identified
Dosing Schedule:	Five times per week
Irradiation Schedule:	Irradiation occurred after test article on Monday, Wednesday and Friday. Irradiation occurred before test article on Tuesday and Thursday.
Duration of Treatment:	40 weeks, followed by a 12 week observation period for the development of tumors

APPEARS THIS WAY
ON ORIGINAL

Doses: refer to study design table below

Study Design

Treatment	Volume (µl/mouse/day)	Dose (mg/kg/day)	UVR Dose (RBU/day)	UVR Dose (RBU/week)
Untreated Control – Low UV	0	0	120	600
Vehicle lotion	100	0	120	600
15% BMS 203522 lotion	10	60	120	600
15% BMS 203522 lotion	30	180	120	600
15% BMS 203522 lotion	100	600	120	600
Untreated Control – High UV	0	0	240	1200

15% BMS 203522 lotion or vehicle lotion 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. 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 Robertson-Berger Units {RBU}).

- *Basis of Dose Selection:*

Dose selection was based on the results of a two week dermal tolerance study in hairless albino mice. The 15% BMS 203522 lotion was well tolerated in that study at a dose volume of 100 µl/mouse/day. The dose volume of 100 µl/mouse/day is the maximum feasible dose volume to apply to the mouse and 15% is the maximum drug concentration feasible in the lotion formulation.

The sponsor submitted the protocol for the photocarcinogenicity study to the division on 3-9-98 after the study was already in progress. My recommendations for the protocol included that it would have been beneficial for the sponsor to have conducted a proper dose range finding study for the photocarcinogenicity study with various concentrations of the BMS 203522 lotion (i.e., ——————15%). The results from such a study would have provided more reliable data for determining the proper doses for the photocarcinogenicity study. However, I noted that since the sponsor had already initiated the study, the selected dose range may be adequate to test for the photocarcinogenic potential of the test article.

- *Relation to Clinical Use:* The intended route in humans is topical administration.

- *CAC Concurrence:* No CAC concurrence was obtained for the doses selected in this study. The sponsor received concurrence from the division on the dose selection and protocol for this photocarcinogenicity study.

- *Restriction Paradigm for Dietary Restriction Studies:* No
- *Route of Administration:* Topical
- *Frequency of Drug Administration:* 1X/day
- *Dual Controls Employed:* No
- *Interim Sacrifices:* No
- *Satellite PK or Special Study Group(s):* No
- *Unscheduled Sacrifices or Deaths:* No
- *Deviations from Original Study Protocol:* None

Study Results and Frequency of Monitoring:

- *Clinical Observations:* Twice daily for viability and weekly for clinical observations.

No treatment related clinical observations were noted in this study.

The vehicle alone and untreated high UV control groups had an increased number of mice sacrificed due to excess tumor burden compared to BMS 203522 lotion treated groups. The number of mice found dead or moribund sacrificed is presented in the following table.

BMS 203522 (mg/kg/day)	UVR Dose (RBU/week)	# of males found dead	# of males moribund sacrificed	Total male mortality	# of females found dead	# of females moribund sacrificed	Total female mortality
Untreated control	600	4	9	13**	2	5	7**
0-vehicle control	600	0	36	36[a]	0	36	36[a]
60	600	3	22	25**	2	13	15**
180	600	4	13	17**	0	14	14**
600	600	0	14	14**	1	13	14**
Untreated control	1200	1	35	36[b]	1	35	36[b]

a – all surviving vehicle control mice were sacrificed during week 49 of the study because of group tumor burden criteria.
b – all surviving untreated control (high UV) mice were sacrificed during week 38 of study because of group tumor burden criteria
** - $p<0.001$ when compared to vehicle control animals.

- *Dermal Observations:* The skin was examined weekly for signs of erythema, flaking, edema, ulceration, skin tumors or other indications of skin toxicity.

Minimal (Grade 1) skin irritation consisting of erythema, edema, flaking and skin thickening was noted at the application sites of all test article and vehicle treated groups. No dose-dependent response was noted for the level of skin irritation noted at the treatment sites.

- *Body Weight:* Body weights were recorded weekly.

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

- *Gross Necropsy:* Gross Necropsy conducted at time of sacrifice.

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

- *Tumor Development:* The locations, sizes and progression history of skin tumors were recorded manually using a grid location system and separate data sheet for each individual test animals. Skin tumor development was evaluated in terms of Prevalence, Median Tumor Onset, Tumor Amplification Factor and Tumor Yield and groups were statistically compared.

Analysis of the onset of UV-induced skin tumors showed a significant reduction in the median number of weeks to onset in the vehicle control group (32 weeks) compared to the low UV control (43 weeks) or the BMS 203522 lotion treated groups (low dose – 42 weeks, mid dose – 41.5 weeks, high dose – 43 weeks). Median tumor onsets were similar between the BMS 203533 lotion treated groups and the low UV control group. As expected, a significantly accelerated median onset occurred in the high UV control group (24.5 weeks).

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

BMS 203522 (mg/kg/day)	UVR Exposure (RBU/Week)	Sexes Combined Median (Weeks)	Male Mice Median (Weeks)	Female Mice Median (Weeks)
Untreated control	600	43.0	46.5	40.5
0-vehicle control	600	32.0	33.5	31.0
60	600	42.0	42.5	42.0
180	600	41.5	43.0	39.5
600	600	43.0	43.0	42.0
Untreated control	1020	24.5	25.0	24.0

Overall Interpretation and Evaluation:

- Adequacy of the carcinogenicity studies and appropriateness of the test model:

The photocarcinogenicity model used for this study is an appropriate animal model for analysis of photo co-carcinogenicity. The dose range study conducted to support the doses selected for this study was deemed adequate.

- Evaluation of Tumor Findings:

Topical administration of the 15% BMS 203522 lotion at dose levels up to 600 mg/kg/day, had no effect on dermal tumors induced by UV radiation. The vehicle formulation alone appeared to enhance photocarcinogenesis when compared to the same dose of UV radiation without any treatment. This effect has been previously reported in the literature with other vehicles[4] and has been noted in other photocarcinogenesis studies submitted to the agency[5].

It is interesting to note that topical administration of the test article significantly reduced UVR induced skin tumor development in comparison to vehicle lotion. This result may provide support that the test article has some potential to protect from photocarcinogenesis. The sponsor submitted two publications to the NDA concerning inhibition of photcarcinogenesis with BMS 203522 administration. The first article reported that eflornithine inhibited UVB-induced carcinogenesis in mice[6]. The second abstract reported that eflornithine suppressed skin tumorigenesis following a 6 month dietary administration in the v-Ha-*ras*/Keratin K1 transgenic mouse model[7].

Summary Conclusions and Recommendations:

- Acceptability of Study(s) or Overall Testing Approach:

The photo co-carcinogenicity study is acceptable because the highest dose tested consisted of the maximum feasible dose volume that can be applied to the back of a mouse and the test article concentration was the maximum feasible concentration for the lotion formulation. The overall testing approach for the photo co-carcinogenicity study in the mouse was the division's accepted model for this study.

- Major Tumor Findings:

Analysis of the onset of UV-induced skin tumors showed a significant reduction in the median number of weeks to onset in the vehicle control group (32 weeks) compared to the low UV control (43 weeks) or the BMS 203522 lotion treated groups (low dose – 42 weeks, mid dose – 41.5 weeks, high dose – 43 weeks). Median tumor onsets were similar between the BMS 203533 lotion treated groups and the low UV control group. As expected, a significantly accelerated median onset occurred in the high UV control group (24.5 weeks).

[4] Kligman LH and Kligman AM. (1992) Petrolatum and other hydrophobic emollients reduce UVB-induced damage. Journal of Dermatological Treatment 3: 3-7.

[5] Jacobs A, Avalos J, Brown P and Wilkin J (1999) Does photosensitivity predict photocarcinogenicity? Int. J. Tox. 18: 191-198.

[6] Gensel HL (1991) Prevention by α-difluoromethylornithine of skin carcinogenesis and immunosuppression induced by ultraviolet irradiation. J. Cancer Res. Clin. Ocol. 117: 345-350.

[7] McCormick DL, Rao KV, Gram TA, Steele VE, Lubet RA, Kellof GJ (1997) Chemoprevention of skin tumorigenesis in the v-HA-*ras*/Keratin K1 transgenic mouse by retinoids and α-difluoromethylornithine. Toxicologist 36: 176.

- Non-neoplastic Findings:

Minimal (Grade 1) skin irritation consisting of erythema, edema, flaking and skin thickening was noted at the application sites of all test article and vehicle treated groups. No dose-dependent response was noted for the level of skin irritation noted at the treatment sites.

- Biological Significance:

No biologically significant increase in photocarcinogenic risk was observed for the BMS 203522 lotion based on the results of this study.

- Potential Clinical Implications of Findings:

BMS 203522 lotion administration does not appear to increase the photocarcinogenic effect associated with UV exposure.

- Recommendations for Further Analysis:

No recommendations for further analysis at this time.

Carcinogenicity Study #2:

BMS-203522 Lotion: Two-Year Dermal Carcinogenicity Study in Mice

Study Title: BMS-203522 Lotion: Two-Year Dermal Carcinogenicity Study in Mice
Study Number: 96701

Note: The dermal carcinogencitiy study conducted with BMS 203522 lotion was reviewed in more detail in an addendum review and will be summarized below.

The highest dose for this dermal carcinogenicity study was based on the maximum feasible concentration (15%) of BMS 203522 in the vehicle and the maximum feasible volume (100 µl) that can be applied to the mouse. The dose groups tested in the study are provided in the following table:

Study Design

Group	Treatment	Dose Level (µl/mouse/day)	Dose Level (mg/kg/day)	Number of Main Study Animals		Number of Toxicokinetic Animals	
				Males	Females	Males	Females
1	Untreated Control	0	0	50	50	0	0
2	Vehicle Control	0	0	50	50	0	0
3	15% BMS-203522	25	150	50	50	42	42
4	15% BMS-203522	50	300	50	50	42	42
5	15% BMS-203522	100	600	50	50	42	42

The test article formulation and vehicle control were administered by dermal application from an ——— pipette once daily, 7 days a week for 2 years. The test article formulation was applied to the clipped anterior dorsum up to the interscapular region of the back. The test article was applied to ~10% of the body surface (~1 x 3 cm area). The dorsal skin of each mouse was prepared for treatment by close clipping of the air with an electric clipper. The application area was clipped throughout the study period as necessary. The test article and vehicle remained on the animals for ~6 hours each day and was removed by wiping with gauze moistened with water. The untreated controls had the shaved area wiped in the same manner.

No treatment related effects were observed in mortality, clinical observations, dermal irritation, body weights, food consumption or gross observations. No treatment related neoplasms occurred systemically or at the skin application sites.

The toxicokinetic analysis showed that the systemic exposure of the mice to BMS 203522 was continuous and dose related in this study. No difference in pharmacokinetic parameters was noted between male and female mice. No accumulation of BMS 230522 was noted over the study period. The toxicokinetic parameters are summarized in the following two tables.

Summary of Toxicokinetic Parameters
(Study Days 1 and 87 measurements)

Dose (mg/kg/day)	Study Day	C_{max} (ng/ml)		T_{max} (hr)		$AUC_{(0\text{-}24\ hr)}$ (ng·hr/ml)	
		Male	Female	Male	Female	Male	Female
150	1	15383	22007	1	1	60482	69623
	87	14941	13681	1	1	37048	37330
300	1	71825	68061	3	3	313770	282174
	87	15652	17100	1	1	52442	65924
600	1	101230	71706	1	1	442619	292739
	87	41702	55844	1	1	137254	215510

Summary of Plasma Concentrations
(Study Days 1, 87 and 367 measurements)

Dose (mg/kg/day)	Study Day	Mean Plasma Conc. at 6 hr (ng/ml)		Mean Plasma Conc. at 18 hr (ng/ml)	
		Male	Female	Male	Female
150	1	2248	2261	90.1	90.5
	87	1024	1137	156.2	125.2
	367	1087	2443	75.4	97.0
300	1	7522	7825	346.4	227.8
	87	1593	4171	170.7	269.9
	367	1924	1942	106.9	250.7
600	1	20008	20796	247.6	443.5
	87	6023	4925	403.8	1920
	367	2324	6122	274.1	328.6

The sponsor has estimated that the maximum human dosing of the 15% BMS 203522 lotion will be 2.5 mg/kg/day for a 50 kg female (92.5 mg/m^2/day). A repeat dose pharmacokinetic clinical study under conditions of maximum use has been conducted for the 15% BMS 203522 lotion. Data concerning this study was obtained from the Clinical Pharmacology reviewer, Taposh Ghosh. In this clinical study, twice daily application of 0.5 g of the 15% BMS 20355 lotion (75 mg BMS 203522/application) was applied to the chin area for seven days in hirsute women. This equals a clinical dose of 3 mg/kg/day (111 mg/m^2/day) for a 50 kg female. The AUC value obtained from the clinical pharmacokinetic study was 185 ng·hr/ml for a daily exposure (AUC = 92.5 ng·hr/ml was reported in the study after a 12 hour exposure, clinical treatment is 2-12 hour exposures per day). The mean AUC value in the mouse dermal carcinogenicity for the high dose group (600 mg/kg/day; 1800 mg/m^2/day) was 176382 ng·hr/ml on day 87. It appears that the AUC value in the dermal carcinogenicity study leveled off at the day 87 timepoint. The systemic exposure obtained in the mouse dermal carcinogenicity study at the high dose level (which showed no evidence of carcinogenicity) is ~950 times the systemic exposure noted in the human clinical pharmacokinetic study conducted under maximum human use for the drug product. This provides for an adequate safety margin for the 15% BMS 203522 lotion concerning systemic carcinogenic potential after topical administration under clinical conditions of use.

The results from this 2 year dermal carcinogenicity study were presented to the Executive CAC on 5/2/00. The Executive CAC recommendations and conclusions are presented below.

Executive CAC Recommendations and Conclusions:

1. The committee determined that the mouse dermal carcinogenicity study was adequate and concurred that the study results were negative for carcinogenicity.
2. The committee recommended asking the sponsor for the starting and ending date for the study (a GLP question).

3. The committee recommended that human AUC values be obtained to calculate fold exposure levels for the mouse dermal carcinogenicity study.

Note: Originally I reported that the study report dates were not stated in the submission. During the Exec CAC presentation of this dermal carcinogenicity study, the Exec CAC recommended that I ask the sponsor for the study dates since this was a GLP issue. Subsequently after through scanning of the final study report, I was able to locate the study dates, which have been presented in the addendum review of the dermal carcinogenicity study.

Note: Human AUC values have been obtained from the Clinical Pharmacology reviewer, Taposh Ghosh, and the fold exposure levels for the dermal carcinogenicity study have been calculated in the section above the Executive CAC recommendations and conclusions.

OVERALL SUMMARY AND EVALUATION:

Introduction:

BMS 203522 (eflornithine, 2-difluoromethylornithine {DMFO}) is an irreversible ornithine decarboxylase inhibitor under development as a topical treatment for female facial hirsutism. The toxicity profile of BMS 203522 has been characterized in several nonclinical *in vitro* and *in vivo* toxicity studies conducted by the sponsor. In addition, DMFO is being investigated by the National Cancer Institute as a potential oral chemopreventive drug. Additional nonclinical toxicity studies were conducted by NCI to support the safety of DMFO for this indication. A brief summary of the significant toxicities and corresponding safety evaluation based on these studies is provided in the following section.

Safety Evaluation:

The chronic systemic toxicity for orally administered DMFO has been characterized in nonclinical toxicology studies (52 week duration) conducted by NCI in rats and dogs. Potential target organs of toxicity identified in these studies included skin, liver, gastrointestinal tract and conjuctiva. It is interesting to note that oral DMFO side effects in humans include seizures, anoxia, gastrointestinal symptoms, anemia thrombocytopenia and decreased hearing acuity. The thrombocytopenia and ototoxicity effects have not been noted in oral nonclinical toxicity studies to date.

Apparently, several genotoxicity studies were conducted for DMFO by NCI. Unfortunately only summary information was included in the submission. This summary information stated that 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 teratogenicity studies were conducted for DMFO by NCI in rats and rabbits. DMFO did not demonstrate any teratogenic potential in these nonclinical reproductive toxicity studies. The maternal NOEL in rats was 200 mg/kg/day and the fetal NOEL was 80 mg/kg/day. The maternal NOEL in rabbits 135 mg/kg/day and the fetal NOEL was 45 mg/kg/day.

The preliminary topical formulations of BMS 203522 evaluated by the sponsor included a [redacted] containing [redacted] BMS 203522 (designated as SP33) and cream formulations containing [redacted] 15% BMS 203522 (designated SP106). The 15% BMS 203522 lotion formulation that was selected for commercial development and tested in several pivotal nonclinical studies, is identical to the 15% SP106 cream formulation, except for the concentration of [redacted]

Acute toxicology studies conducted for topical BMS 203522 formulations included a single dose dermal study in rabbits and a single dose oral study in rats. In the single dose dermal study in rabbits, 5000 mg/kg of the [redacted] BMS 203522 (SP33) formulation under 24 hour occlusion was well tolerated producing only minimal skin irritation. In the single dose oral study in rats, 1500 mg/kg of the 15% BMS 203522 (SP106) formulation was well tolerated and a minor toxicity noted in this study was a transient, low incidence of soft stools. The [redacted] BMS 203522 (SP33) and 15% BMS 203522 (SP106) formulations can be characterized as slightly toxic following single dermal or oral administration, respectively, based on the results of the acute toxicology studies.

Several nonclinical dermal irritation studies were conducted in hamsters and rabbits. In a 12 day dermal irritation study in hamsters, 10 µl/animal doses of the [redacted] 15% BMS 203522 (SP106) formulations were well tolerated with no dermal effects noted in this study. A series of 5, 14 and 21 day dermal irritation studies were performed in rabbits. In the 5 and 14 day studies, [redacted] BMS 20355 (SP33), [redacted] 15% BMS 203522 (SP106) formulations and the SP106 vehicle produced mild to moderate skin irritation when applied under occluded or unoccluded conditions. In the 21 day rabbit dermal irritation study, 15% BMS 203522 (SP106) and the SP106 vehicle were nonirritating when given once daily or mildly irritating when given twice daily at 80 µl/application with no occlusion. However, in a 26 week dermal toxicity study in rabbits, the [redacted] 15% concentrations of BMS 203522 (SP106) formulation and the SP106 vehicle produced marked dermal irritation at a dose volume of 125 µl/application administered twice daily. The level of dermal irritation was severe enough to warrant discontinuation of the study after day 11. The results of this study suggested that higher volumes of the 15% BMS 203522 (SP106) formulation were significantly irritating to the skin of rabbits after repeat dose administration. Therefore, it was determined that another non-rodent species (minipig) would be more suitable for the long-term dermal toxicity study of 15% BMS 203522 cream.

Special toxicity studies were performed to determine the ocular irritation potential in rabbits and the phototoxicity and sensitization potential in guinea pigs of BMS 203522 topical formulations. The 15% BMS 203522 cream (SP106) formulation was not classified as an eye irritant in rabbits. However the [redacted] BMS 203522 [redacted] (SP33) formulations was classified as an eye irritant in rabbits. The irritation was reversible and considered to be related

to the ——— vehicle. The — BMS 203522 ——— (SP33) formulation was non-sensitizing in guinea pigs. It is important to note that typically it is requested that the nonclinical sensitization study be conducted with the to be marketed formulation. However, clinical sensitization studies have been conducted with the 15% BMS 203522 lotion (to be marketed formulation). The results demonstrated that 15% BMS 203522 lotion was non-sensitizing in humans. Therefore, it will not be requested of the sponsor to repeat the nonclinical sensitization study. The 15% BMS 203522 cream (SP106) formulation was not phototoxic in guinea pigs under the conditions of the study. It is important to note that this was not a GLP study and that only UVA exposure was administered in the nonclinical photoxicity study. The BMS 203522 cream (SP106) formulation UV spectrum demonstrates absorption in the UVB range. Therefore, the nonclinical phototoxicity study should have been conducted with UVA and UVB exposure. However, clinical phototoxicity studies have been conducted with the 15% BMS 203522 lotion (to be marketed formulation) under solar simulated (UVA/UVB/Vis) exposure conditions. The results demonstrated that the 15% BMS 203522 lotion was not phototoxic in humans. Therefore, it will not be requested of the sponsor to repeat the nonclinical phototoxicity study.

Long term dermal toxicity studies for BMS 203522 topical formulations were conducted in rats and minipigs. In the 26 week dermal toxicity study in rats, 45 µl/application (2X/day) of either — BMS 203522 ——— (SP33) formulation or 15% BMS 203522 cream (SP106) formulation was applied dermally, 7 days/week. No treatment related toxic or irritant effects were noted in rats under the conditions of this study. Evaluation of plasma samples obtained at terminal sacrifice (~18 hours after the last dose during week 26) showed no detectable systemic exposure to BMS 203522. Based on the results of this study, the NOAEL in rats administered topical BMS-203522 cream daily for 26 weeks was 15% BMS 203522 cream (90 µl/day of SP106 formulation; ~68 mg/kg/day or ~400 mg/m^2/day for a 200 g rat). The NOAEL in this study is ~4 times the maximum human dose (400 mg/m^2/day ÷ 92.5 mg/m^2/day).

In the 52 week dermal toxicitiy study in minipigs, the 15% BMS 203522 lotion formulation at daily dermal doses of 15 and 150 mg/kg/day was well tolerated with no evidence of systemic or dermal toxicity. The 150 mg/kg/day dose was the maximum feasible dose in minipigs based on the maximum dose volume that could be applied (0.5 ml/kg, 2X/day) and the maximum concentration achievable with the formulation (15%). Based on the results of this study, the NOAEL in minipigs administered topical BMS-203522 lotion daily for 52 weeks was 15% BMS 203522 lotion (150 mg/kg/day or 5250 mg/m^2/day). The NOAEL for systemic effects in this study is ~57 times the maximum human dose (5250 mg/m^2/day ÷ 92.5 mg/m^2/day).

A battery of reproductive and developmental toxicity studies were conducted for BMS 203522. In the dermal fertility and early embryonic development study in rats, the 15% BMS 203522 lotion at doses of 50, 150 or 450 mg/kg/day produced dose related dermal irritation. No treatment related systemic toxicity or effects on reproductive or fertility indices were noted in this study. Based on the results of this study, the NOAEL in rats for systemic maternal and reproductive toxicity is 450 mg/kg/day (2700 mg/m^2/day). The NOAEL in this study is ~29 times the maximum human dose (2700 mg/m^2/day ÷ 92.5 mg/m^2/day).

In the first dermal embryo-fetal development toxicity study in rats, the 15% BMS 203522 cream (SP106) formulation at daily doses of 225 and 450 mg/kg/day resulted in reduced maternal body weight gains during the later part of gestation. Mean food consumption was lower in high dose animals. No dermal irritation was noted in this study. Pregnancy rats were 100% for all dose groups. However, the percentages of live fetuses were 93.3, 25.4 and 5.4% for control, low and high dose groups, respectively. In utero growth retardation, as evidenced by decreased fetal weights and delayed ossification and development of viscera, was noted in both the low and high dose groups. Unfortunately neither a maternal or developmental NOAEL could be established in this study. Both the maternal and developmental NOAELs were less that 225 mg/kg/day based on the results of this study. The sponsor attributes the results of this study to the fact that no precautions were taken to prevent access of the dams to the drug by covering the application sites or collaring the rats. Evaluation of drug levels in maternal blood on days 15 and 19 of gestation showed plasma drug levels that were higher than expected on the basis of previous dermal absorption data. Therefore, the sponsor proposed that it is likely that ingestion of the drug occurred during the study and the adverse maternal and developmental effects may have resulted from oral, and not dermal, exposure. The sponsor elected to repeat the dermal rat teratogenicity study with appropriate precautions.

A second dermal embryo-fetal development study in rats was performed with the 15% BMS 203522 lotion at daily doses of 90, 225 and 450 mg/kg/day. In this study, animals were collared and the application sites were covered continuously during the dosing period to prevent ingestion of BMS 203522 by dams. Levels of BMS-203522 in maternal plasma in this study compared to the previous study show a reduced extent of absorption of the test article after dermal application. The circulating plasma levels of BMS-203522 were 11 to 14 fold higher in the previous study compared with this study. The presence of a dose dependent erythema in this study in mid and high dose group animals, that was not seen in the previous study, provides additional evidence that ingestion of the test article may have occurred in the previous study. The results of this study indicate that topically applied 15% BMS 203522 lotion is not teratogenic or embryolethal to rats under the conditions of this study. The maternal NOAEL was this study was 90 mg/kg/day (540 mg/m^2/day; 10 $\mu l/cm^2$) under the conditions of this study. The fetal NOAEL was 450 mg/kg/day (2700 mg/m^2/day; 50 $\mu l/cm^2$) under the conditions of this study. The maternal NOAEL in this study is ~6 times the maximum human dose (540 mg/m^2/day ÷ 92.5 mg/m^2/day). The fetal NOAEL in this study is ~29 times the maximum human dose (2700 mg/m^2/day ÷ 92.5 mg/m^2/day).

A dermal embryo-fetal development study in rabbits was performed with the 15% BMS 203522 lotion at daily doses of 30, 90 or 300 mg/kg/day. A test article and vehicle related dermal irritation was noted in this study. Marked maternal and fetal toxicity occurred in the high dose group, which consisted of severe dermal irritation, significantly reduced body weight and food consumption, abortions and maternal mortality, increased fetal resorptions and reduced fetal weight. All animals wore Elizabethan collars and had the skin sites covered in this study. However, some rabbits in each group chewed, partially removed or removed the topical bandages, especially in the vehicle control and high dose groups. In addition, high dose animals may have experienced an increased dermal absorption through severely irritated skin. Therefore,

it is possible that increased systemic exposure to BMS 203522 above what would be expected by the dermal route may have occurred in high dose animals. Unfortunately, no pharmacokinetic data was obtained in this study to provide data to support this theory. Topically applied BMS 203522 lotion did not exhibit any teratogenic potential in rabbits under the conditions of this study. The maternal and developmental NOAEL were 90 mg/kg/day (990 mg/m^2/day, 0.6 ml/kg) under the conditions of this study. The maternal and fetal NOAELs in this study are ~10 times the maximum human dose (990 mg/m^2/day ÷ 92.5 mg/m^2/day).

An oral perinatal and postnatal study in rats was performed with BMS 203522 administered in the drinking water at daily doses of 223, 625 and 1698 mg/kg. No adverse effects on maternal reproductive parameters were noted in this study. However, mild toxicity in dams and pups were noted in the mid and high dose groups, which included decreased maternal body weight, food and water consumption, and reduced pup weights. No significant effects on behavior, development or reproductive function of the F_1 offspring were noted in this study. The systemic maternal, neonatal and developmental NOAEL for BMS 203522 was 223 mg/kg (1338 mg/m^2/day) under the conditions of this study. The systemic maternal, neonatal and developmental NOAELs in this study are ~14 times the maximum human dose (1338 mg/m^2/day ÷ 92.5 mg/m^2/day). It is important to note that the fold factor calculated for this study may actually be considerably greater since BMS 203522 is will absorbed orally in rats (78% of a 40 mg/kg dose), whereas minimal absorption occurs in humans treated topically with 15% BMS 203522 lotion (~0.80% of dose; 2X/day treatment for 7 days).

It is recommended that Pregnancy category C is the appropriate pregnancy category for the 15% BMS 203522 lotion. This recommendation is based on the results of the dermal teratogenicity studies conducted in rats and rabbits and the low level of systemic absorption (~0.80%) noted after 7 day repeat administration of the maximum dose of the 15% BMS 203522 (twice/day) in humans. Additional recommendations for the inclusion of potential reproductive toxicity information in the label will be described in more detail in the Labeling Review section below.

A battery of genotoxicity tests were conducted for BMS 203522. BMS 203522 was not genotoxic in the ICH standard battery of genotoxicity assays (Ames test, chromosomal aberration assay and dermal rat micronucleus assay). The rat micronucleus test was conducted by the dermal route for the test article. It would have been advisable to conduct the rat micronucleus assay via the systemic route to increase the systemic exposure to the test article in the rat micronucleus assay. However, the high dose of 15% BMS 203522 lotion tested in the dermal rat micronucleus assay is ~58 fold greater than the maximum daily human dose. This is an acceptable safety margin for the dermal rat micronucleus assay.

A photocarcinogenicity study and dermal carcinogencity study were conducted in mice. The photocarcinogenicity study in mice was performed with the 15% BMS 203522 lotion at daily doses of 60, 180 and 600 mg/kg/day. No test article effect on dermal tumorigenicity caused by UVA/UVB radiation was noted in this study. In contrast, the vehicle formulation alone appeared to enhance photocarcinonensis when compared to the same dose of UV radiation

without any treatment. This has been previously reported in the literature with other vehicles. The results of this study indicate that the 15% BMS 203522 lotion does not have a increased photocarcinogenic risk associated with its use under the conditions of this study.

The dermal carcinogenicity study in mice was performed with the 15% BMS 203522 lotion at daily doses of 150, 300 and 600 mg/kg/day. No treatment related effects were observed in mortality, clinical observations, dermal irritation, body weights, food consumption or gross observations. No treatment related neoplasms occurred systemically or at the skin application sites. The high dose in both the photocarcinogenicity and dermal carcinogenicity studies was the maximum feasible dose [maximum dose volume that can be applied to a mouse (100 µl) and maximum feasible concentration of BMS 203522 in the formulation (15%)].

A repeat dose pharmacokinetic clinical study under conditions of maximum use has been conducted for the 15% BMS 203522. Data concerning this study was obtained from the Clinical Pharmacology reviewer, Taposh Ghosh. In this clinical study, twice daily application of 0.5 g of the 15% BMS 20355 lotion (75 mg BMS 203522/application) was applied to the chin area for seven days in hirsute women. This equals a clinical dose of 3 mg/kg/day (111 mg/m^2/day) for a 50 kg female. The AUC value obtained from the clinical pharmacokinetic study was 18[illegible] ng·hr/ml for a daily exposure (AUC = 92.5 ng·hr/ml was reported in the study after a 12 hou[illegible] exposure, clinical treatment is 2-12 hour exposures per day). The mean AUC value in the mouse dermal carcinogenicity for the high dose group (600 mg/kg/day; 1800 mg/m^2/day) was 176382 ng·hr/ml on day 87. It appears that the AUC value in the dermal carcinogenicity study leveled off at the day 87 timepoint. The systemic exposure obtained in the mouse dermal carcinogenicity study at the high dose level (which showed no evidence of carcinogenicity) is ~950 times the systemic exposure noted in the human clinical pharmacokinetic study conducted under maximum human use for the drug product. This provides for an adequate safety margin for the 15% BMS 203522 lotion concerning systemic carcinogenic potential after topical administration under clinical conditions of use.

Clinical Relevance of Safety Issues:

The potential toxicity of BMS 203522 has been adequately studied in nonclinical toxicology studies. No significant nonclinical toxicity findings were noted that would preclude the use of the 15% BMS 203522 lotion in the treatment of female facial hirsutism.

Conclusions:

Based on the nonclinical data available for 15% BMS 203522 lotion, my recommendation for NDA 21-145 is that it be approvable from a pharmacology/toxicology perspective provided that the recommended changes in the label discussed in the next section are incorporated into the label.

Labeling Review:

The entire Vaniqa® label is inserted below. Comments about the portions that relate to nonclinical pharmacology/toxicology will be inserted directly in the appropriate sections. Recommended sections to be deleted are marked by ~~strikeout.~~ Recommended sections to be added are marked by highlight.

DRAFT PACKAGE INSERT
May 23, 2000