

Reviewer: Chopra

NDA No. 22-291

GSK Reference No. G07166/ CD2007/01153/00

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Testing Facility: _____

Date of Start & Completion: August 27, 2007 & 15 January 2008

GLP Compliance Statement: A GLP compliance statement with Food and Drug Administration (US) and Pharmaceutical Affairs Bureau, Ministry of Health, Labor and Welfare of Japan was included.

Methods & Materials:

SB-497115-GR (Batch Number TPO-E-02C.031002901) was administered for 84 consecutive days in 6 groups of mice. Mice in groups 1, 3 and 5 were B6C3F1 (pigmented) female mice and mice in groups 2, 4 and 6 were CD-1 (albino) female mice. These were administered SB-497115 and exposed to solar-simulated ultraviolet radiation (UVR: 0.6 MED a maximum feasible UVR dose which did not cause retinal lesions - based on the data from a previous study) as outlined in the study design table below.

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Group	Strain	Number of Mice Main Study/ Toxicokinetic	Dose (mg/kg/day)	UVR Exposure (RBU/Week)
1	B6C3F1	20 ^a 15 ^b	0	1200
2	CD-1	20 ^a 15 ^b	0	1200
3	B6C3F1	40 ^a 15 ^b	100	1200
4	CD-1	40 ^a 15 ^b	150	1200
5	B6C3F1	35 ^a 15 ^b	100	0
6	CD-1	35 ^a 15 ^b	150	0

a. Fifteen mice from main study chosen for Study Days 84-85 toxicokinetic evaluation
b. Mice specifically assigned to Study Days 1-2 toxicokinetic evaluation.

The compound formulations or vehicle [2% (w/v) hydroxypropylmethylcellulose (HPMC E5) with 0.2% (w/v) sodium lauryl sulfate was administered daily oral gavage dose (volume 10 ml/kg). The control groups mice (groups 1 and 2) were given the vehicle formulation and group 3 (B6C3F1 mice) were administered 100 mg/kg/day and group 4 (CD-1 mice) were administered 150 mg/kg/day SB-497115. In addition, 6 TK groups of (15/group) mice were also included. All mice were housed in a room with standard cool white fluorescent lighting. All mice in Groups 1 through 4 were exposed 5 days/week for approximately 36 minutes from Days 1 through 82. From day 83 to 240, the animals were given 3 minimal erythema doses/week or 0.6 minimal erythema doses/day of solar simulated UVR or 1200 RBU/week and evaluated for cataract using slit-lamp biomicroscopy and ophthalmoscopy before assignment and on study days 29, 35, 49, 63 and 78. The mice of main study were killed on days 84 and 85 and the eyes were retained (left eye fixed in Davidson's solution and retained in 70% ethanol, right eye frozen at <-60°C. Blood samples from TK groups were collected on day 1 to 2 and 84 to 85 from both strains of mice administered.

Results:

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1. General Observations: All of the B6C3F1 (pigmented) mice showed no significant adverse clinical observations or deaths due to SB-497115 administration. Six CD-1 (albino) mice included in 150 mg/kg/day SB-497115 treatment group died and adverse effects observed before death were moderate dehydration, labored breathing, decreased motor activity, cold to touch, ptosis, hunched posture, rales, hyperpnea, tremors, lacrimation, chromodacryorrhea, pale pinnae, tachypnea, scant feces, low carriage and lost righting reflex.

2. Toxicokinetics: The mean plasma concentrations at steady state (AUC_{0-t}) were similar in 150 mg/kg/day CD-1 mice and 100 mg/kg/day treated B6C3F1 mice dosed on Day 1 and Day 84 with or without UVR exposure. A summary of toxicokinetics data is shown in sponsor's table below:

Parameter	Period	B6C3F1 (Pigmented) Mice	
		Dose of SB-497115 (mg/kg/day)	
		100	150
AUC_{0-t} ($\mu\text{g}\cdot\text{h}/\text{mL}$)	Day 1	1003	NA
	Day 84	Group 3 ^a	1091
		Group 5 ^b	973
C_{max} ($\mu\text{g}/\text{mL}$)	Day 1	128	NA
	Day 84	Group 3 ^a	123
		Group 5 ^b	120
$T_{1/2}$ (h)	Day 1	4.00	NA
	Day 84	Group 3 ^a	4.00
		Group 5 ^b	2.00
Parameter	Period	CD-1 (Albino) Mice	
		Dose of SB-497115 (mg/kg/day)	
		100	150
AUC_{0-t} ($\mu\text{g}\cdot\text{h}/\text{mL}$)	Day 1	NA	947
	Day 84	Group 4 ^a	1568
		Group 6 ^b	1757
C_{max} ($\mu\text{g}/\text{mL}$)	Day 1	NA	122
	Day 84	Group 4 ^a	128
		Group 6 ^b	158
$T_{1/2}$ (h)	Day 1	NA	4.00
	Day 84	Group 4 ^a	4.00
		Group 6 ^b	4.00

a. Animals in this group were exposed to UVR.
b. Animals in this group were not exposed to UVR.
NA: Not Applicable

3. Ophthalmic Assessment: The cataract was developed in B6C3F1 (pigmented) and CD-1 (albino) mice on day 35 and 49, respectively and the severity being more in mice not exposed to UVR (Groups 5 and 6). In B6C3F1 mice, the primary difference in the two groups (Groups 3 and 5) was the severity of cataractous changes and in CD-1 mice (Groups 4 and 6), the number of mice showing effect to UV exposure were greater than non-exposed animals. Exposure to solar-simulated UVR did not increase the incidence or severity of cataractous changes in B6C3F1 mice given SB-497115. In CD-mice, the overall incidences and progression of cataracts was more from day 48 to the termination of the study. The UVR exposure decreased incidences as seen below.

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Table 1 Incidence (%) of cataracts in B6C3F1 and CD-1 mice given SB-497115 with and without UVR exposure

SB-497115 mg/kg/day	0	B6C3F1		0	CD-1	
Group	1	3	5	2	4	6
UVR	Yes	Yes	No	Yes	Yes	No
Study Day						
29	0	0	0	0	0	0
35	0	0	3	0	0	0
49	0	97	100	0	13	53
63	0	100	100	0	46	88
78	0	100	100	0	47	84

Percent of surviving mice in each group at each time point with any type of cataract.

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Study Type: Repeat Dose Toxicity				Batch No.: TPO-E-02C.031002901		
Daily Dose SB-497115 (mg/kg/day) and UVR Exposure	B6C3F1			CD-1		
	0 (Yes)	100 (Yes)	100 (No)	0 (Yes)	150 (Yes)	150 (No)
Day 35 of Study						
Number of animals evaluated	19	39	34	18	38	33
Number of animals with cataracts ^a	0	0	1	0	0	0
- anterior and/or posterior	0	0	1*	0	0	0
- anterior and/or posterior plus fundus not visualized in one or both eyes	0	0	0	0	0	0
- complete	0	0	0	0	0	0
Day 49 of Study						
Number of animals evaluated	18	39	34	18	38	32
Number of animals with cataracts ^a	0	38	34	0	5	17
- anterior and/or posterior	0	33	16	0	4	6
- anterior and/or posterior plus fundus not visualized in one or both eyes	0	5	18	0	1	3
- complete	0	0	0	0	0	8 ^c
Day 63 of Study						
Number of animals evaluated	18	39	34	18	37	32
Number of animals with cataracts ^a	0	39	34	0	17	28
- anterior and/or posterior	0	1	0	0	8	9
- anterior and/or posterior plus fundus not visualized in one or both eyes	0	35	22	0	3	4
- complete	0	3	12	0	6	15

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Study No. AFA00548
GSK Reference No. G07166

Study Type: Repeat Dose Toxicity				Batch No.: TPO-E-02C.031002901		
Daily Dose SB-497115 (mg/kg/day) and UVR Exposure	B6C3F1			CD-1		
	0 (Yes)	100 (Yes)	100 (No)	0 (Yes)	150 (Yes)	150 (No)
Day 78 of Study						
Number of animals evaluated	18	39	34	18	36	31
Number of animals with cataracts ^a	0	39	34	0	17	26
- anterior and/or posterior	0	1	0	0	1	4
- anterior and/or posterior plus fundus not visible in one or both eyes	0	0	0	0	5	0
- complete	0	38	34	0	11	22
^a Anterior and/or posterior capsular, nuclear and/or complete cataract in one or both eyes. If anterior and/or posterior capsular in one eye and complete in the other eye of same animal, incidence reported as the more severe cataract (i.e. complete). ^b Anterior and equatorial capsular cataract (right eye) observed for animal 1587. ^c Nuclear cataract (both eyes) observed for animal 1461.						

In summary, SB-497115 at a single oral dose of 100 mg/kg/day in B6C3F1 female mice was non-lethal and a 150 mg/kg/day dose in CD-1 (albino) female mice was lethal to 6 mice. The incidences of cataract among B6C3F1 pigmented mice were similar in exposed and non-exposed repeated doses of solar-simulated UVR. The number of cataract among CD-1 mice given 150 mg/kg/day SB-497115 exposed to repeat doses of solar-simulated UVR were lesser than non-exposed animals. B6C3F1 mice given SB-497115 with or without UVR exposure were more sensitive than CD-1 mice and SB-497115 showed a tendency to reduce SB-407115-induced cataract.

2. Determination of Skin Irritation Potential using the Synthetic Reconstituted Human Epidermal Model:

Study #: ED2006/00079/00; 1127/1254

Study Initiation date: 04 July 2006 and 07 July 2006.

Materials and Methods:

The skin irritation potential of the SB-497115 (batch # F081604) was assessed using the _____ model _____ and compared with Triton X-100. The model consisted of an _____

_____ Test materials were applied directly to the culture surface at air interface for treatment periods of 4 and 24 hours. The compound if an irritant, was able to penetrate the stratum corneum of the _____ model and produced cytotoxicity and cell death in the cell layers. The cytotoxicity produced by the

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positive agent MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) to formazan by viable cells in the test material was used as positive and the test compound cytotoxicity compared with MTT.

Results: The relative mean tissue viabilities (% of the negative control), obtained after 4 hour and 24 hour exposures, were 99.7% and 94.9% , respectively (Table # 3 below). No irritant action was observed, therefore, no tissue histology or analysis of inflammatory mediator levels was done.

SB-497115-GR : DETERMINATION OF SKIN IRRITATION POTENTIAL USING THE MODEL

Table 3 Qualitative Evaluation of Tissue Viability (MTT uptake visual evaluation)

Material	Exposure Time			
	4 Hours		24 Hours	
Negative control Untreated tissues	-	-	-	-
Test Material	-	-	-	-
Positive Control Triton X-100 0.1% w/v	na	na	+	+

MTT Visual Scoring Scheme of Skin/Ethnic Tissues

- - Blue tissue (viable)
- + - Blue/white tissue (semi-viable)
- na - Not applicable

SB-74116 application did not affect the mean tissue viabilities and was considered as non-irritant. No tissue histology or analysis of inflammatory mediator levels were done.

3. Determination of Eye Irritation potential using in Vitro Test Strategy: (Study #ED2007/00029/00)

Study Initiation date: 26 July 2006 and 12 October 2006.

Materials and Methods:

The purpose of this study was to determine the eye irritation potential by using the _____ model _____ following treatment periods of 10 and 60 minutes. If the compound was irritant, it will penetrate the corneal epithelial tissue and produce cytotoxicity and cell death.

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The issues treated with 30 mg SB-497115 for 10 min or 60 min and incubated at 37°C. The relative mean color corrected viability of the test material treated tissues was 112.0% after a 10 minute exposure and 106.9% after a 60 minute exposure. b(4)

The compound was irritant following 60 min exposure in vitro tissue but the confirmatory test was not conducted/submitted.

4. In vitro Hemolysis and Precipitation Testing in Rabbit Blood: (Study # CD2003/0112/00; 103272).

This in vitro whole blood assay was done to determine the hemolytic potential of SB-497115-GR on rabbit erythrocytes in three different intravenous formulations. In vitro precipitation was done in rabbit serum (300 ul rabbit serum), 300 µL of 0.9% sodium chloride (negative control), 10 mg/mL saponin (positive control), deionized water (system control), vehicle [5% dextrose, USP with 0.2% Polyvinyl-Pyrrolidone (PVP-40), pH 8 or 5% dextrose, USP with 1% Polyvinyl-Pyrrolidone (PVP-40), pH 8 or 5% dextrose, USP with 1% Polyvinyl-Pyrrolidone (PVP-40), pH 9] or SB-497115-GR at concentrations of 0.23, 0.38, 1, 2, and 3 mg/ml in vehicle. Following 10 min incubation at 37°C and centrifugation, the hemoglobin concentration (evidence of dark red/brown particulate matter consistent with the appearance of the drug substance) of each supernatant was determined using the cyanmethemoglobin method. Percent hemolysis was determined for each test agent. Precipitation was evaluated for vehicle and drug in vehicle.

Results:

SB-497115-GR at >.01 mg/ml in 5% dextrose with 0.2% PVP-40, pH 8 or vehicle alone (5% dextrose with 0.2% PVP-40, pH 8, and 5% dextrose with 1% PVP-40, pH 8 or 9) produced no hemolysis but higher concentrations of ≥1.0 mg/ml SB-497115-GR in whole blood produced hemolysis. SB-497115-GR at concentrations ≤3 mg/ml in vehicle or vehicle alone produced no evidence of precipitation when added to rabbit serum.

SB-497115-GR produced hemolysis in whole rabbit blood but no precipitation rabbit serum.

5. SB-497115-GR: Rabbit Eucleated Eye test: (Study #ED2006/00080/00)

Batch #: 81604

Material and Methods: Three eyes from donor rabbits were treated with the compound and, two additional untreated eyes constituted as control. The clamp/eye was then placed horizontally into a Petri dish and 70 mg (in 0.1 ml) was sprinkled as evenly as possible over the surface of the cornea. After ten seconds the test compound washed off with 20 ml of saline solution and treated eye was returned to the chamber and the saline drip repositioned to irrigate the eye. The untreated eyes were similarly washed and used for control purposes.

Assessment of corneal cloudiness was made as pre-enucleation, post equilibration and approximately 60, 120, 180 and 240 minutes following treatment. The thickness of the cornea was measured using an ultrasonic pachymeter and facilitated by a slit-lamp biomicroscope. The endpoint assessed are:

REET Parameter*	REET Cut-Off Value
Maximum Corneal Opacity (Corneal Cloudiness x Area)	> or = 4
Maximum Fluorescein Uptake (Intensity x Area)	> or = 4
Mean Corneal Swelling (mins): 60, 120, 240	> or = 25%
Corneal Epithelium Observations	Any with pitting, mottling or sloughing

Corneal swelling of the test eyes during the study period was considerably greater than that observed in the control eyes over the same period.

Table 3 Determination of Corneal Swelling (%)

Test Eyes											
Chamber Number	Observation Period (mins)	Mean Corneal Thickness (µm)	Corneal Swelling (%) ^a	Chamber Number	Observation Period (mins)	Mean Corneal Thickness (µm)	Corneal Swelling (%) ^a	Chamber Number	Observation Period (mins)	Mean Corneal Thickness (µm)	Corneal Swelling (%) ^a
1	Post equilibration	352.8	N/A	3	Post equilibration	395.0	N/A	5	Post equilibration	357.4	N/A
	60 Post treatment	412.2	16.8		60 Post treatment	473.6	19.9		60 Post treatment	424.8	18.9
	120 Post treatment	438.0	24.1		120 Post treatment	499.0	26.3		120 Post treatment	458.6	28.3
	180 Post treatment	464.2	31.6		180 Post treatment	530.6	34.3		180 Post treatment	490.0	37.1
	240 Post treatment	476.4	35.0		240 Post treatment	561.6	42.2		240 Post treatment	498.4	39.5
Control Eyes*											
Chamber Number	Observation Period (mins)	Mean Corneal Thickness (µm)	Corneal Swelling (%) ^a	Chamber Number	Observation Period (mins)	Mean Corneal Thickness (µm)	Corneal Swelling (%) ^a	Test Eyes			
2	Post equilibration	362.8	N/A	4	Post equilibration	400.4	N/A	Mean corneal swelling 1 hour following treatment 18.5%			
	60 Post treatment	360.4	0.0		60 Post treatment	422.4	5.5	Mean corneal swelling 2 hours following treatment 25.3%+			
	120 Post treatment	352.2	0.0		120 Post treatment	419.6	4.8	Mean corneal swelling 4 hours following treatment 38.9%+			
	180 Post treatment	349.0	0.0		180 Post treatment	413.2	3.2	Control Eyes			
	240 Post treatment	357.0	0.0		240 Post treatment	409.8	2.3	Mean corneal swelling 1 hour following treatment 2.7%			
								Mean corneal swelling 2 hours following treatment 2.4%			
								Mean corneal swelling 4 hours following treatment 1.2%			

SB-497155 was a strong rabbit eye irritant in the test.

6. SB-497115-GR: Local Lymph Node Assay in the Mouse — Project #1127/1257

Testing Facility: _____

Methods: In this study, the skin sensitization potential of the compound was assessed in the CBA/Ca strain mouse following topical application on the dorsal ear surface. Three groups (4/group) of mice

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were treated with 50 µl (25 µl per ear) of the test material (batch #F081604) as a suspension in acetone/olive oil 4:1 at concentrations of 5%, 15% or 50% w/w. An additional group of four animals was treated with acetone/olive oil 4:1 alone.

The proliferation response of lymph node cells was expressed as the number of radioactive disintegrations per minute per lymph node (dpm/node) and as the ratio of ³HTdR incorporation into lymph node cells of test nodes relative to that recorded for the control nodes (Stimulation Index). The test material was a sensitizer if at least one concentration of the compound results in a threefold or greater increase in ³H-TdR incorporation compared to control values. Any test material failing to produce a threefold or greater increase in ³H-TdR incorporation will be classified as a "non-sensitizer".

Results. The Stimulation Index (SI) expressed as the mean radioactive incorporation for each treatment group divided by the mean radioactive incorporation of the vehicle control group and the results are shown in the table below:

Concentration (% w/w) in acetone/olive oil 4:1	Stimulation Index (SI)	Result
5	1.38	Negative
15	1.05	Negative
50	0.84	Negative

The test material did not increase in the incorporation of radioactivity and was considered to be a non-sensitizer under the test conditions.

7. Phototoxicity:

Eltrombopag absorbs light in this spectrum with a peak at 413 nm. Single and repeat dose in vivo tissue distribution studies with [¹⁴C]eltrombopag in pigmented rats indicated that eltrombopag did not accumulate in the lens and not selectively retained in skin or eye of these animals. In an in vitro phototoxicity study (3T3 fibroblast neutral red uptake assay), eltrombopag was toxic (IC₅₀ value of 0.543 µg/mL) in the presence of ultraviolet-A (UV-A).

a. Tolerability, Toxicokinetics and Phototoxic Potential of orally administered eltrombopag in —.SKH1-hr Hairless Mice: (Study #CD2006/0167/100; GSK#D06264) b(4)

Conducted Laboratory: _____ Plasma analyses TK evaluation Laboratory: GlaxoSmithKline, King of Prussia, PA, USA. b(4)

In this study, the tolerability, toxicokinetics and phototoxic potential of orally (via gavage) administered eltrombopag (batch # not provided) was assessed in male and female —.SKH1-hr hairless mice treated for 14 days followed by a single exposure to radiation from a xenon lamp (to simulate sunlight). An area approximately 1.3 cm² of the dorsum of each mouse was exposed to ultraviolet radiation (UVR - a 6.5 kW long-arc xenon water-cooled lamp) to simulate mid latitude summer sunlight. The mouse located approximately 1.2 meters from the UVR source was exposed b(4)

equivalent to approximately 0.5 minimal erythema dose (MED) in approximately 30 minutes. MED refers to a UVR dose adequate to elicit a barely perceptible response in skin and is equivalent to an instrumental dose of approximately 100 J/m². The doses were selected based on preliminary results of 2 year carcinogenicity study in which male and female CD-1 mice treated with the doses of 25, 75, 150 and 300 mg/kg/day [GSK #CD2006/00751/00]. A dose of 300 mg/kg/day was lethal as all animals in this group were terminated during week 2 and 150 mg/kg/day dose was associated with excessive mortality at week 2 and 3 in females resulting in a reduction in dose to 115 mg/kg/day during week 21 of test article administration. But in a subsequent 12 weeks oral toxicity study in female CD-1 mice, a dose of 150 mg/kg/day was tolerated for [GSK #CD2006/00477/00]. Based on this information, the doses of 25, 75 and 150 mg/kg/day were selected. The animals were observed daily and changes in body weights estimated. Skin observations were recorded prior to UVR exposure on Day 14, and three days following UVR exposure approximately 1 hour ± 10 minutes and 4 hours ± 30 minutes after the completion of UVR exposure. After the 3-day post-UVR observation period, main study mice were euthanized on day 17 and the UVR-exposed skin site was collected and microscopic examination was performed. Toxicokinetic sampling (3 mice/sex/timepoint) was performed for satellite groups of test article-treated mice at 0 (pre-dose), 1, 2, 4, 8 and 24 hours after administration of the test article formulations on day 14 and mice were discarded after TK estimation.

Results:

Toxicokinetic analysis showed that SB-497115 was absorbed in male and female mice at the time of UVR exposure (120 ± 10 minutes after the final dose on day 14 of the study). One female main study mouse of 25 mg/kg/day was killed on day 8 due to labored breathing, decreased motor activity, cold to touch, hunched posture and discolored (purple) and swollen left side of the neck. Necropsy revealed red gelatinous material present subcutaneously on the left side of the neck, in the thoracic cavity, along the entire length of the trachea and in both axillae. In addition, there were dark red nodules adhered to the trachea and esophagus. The death of the animal was considered due gavage error and considered not due to test article-related by sponsor. All other main study and toxicokinetic mice survived to scheduled termination and no adverse skin responses were noted during the three days following UVR exposure. There were no test article-related clinical signs.

Slight mean body weight loss up to 4% in males given ≥75 mg/kg/day or greater dose and, 5% for females given 150 mg/kg/day was reported on day 14 when compared to respective pretreatment day 1. SB-497115 at oral gavage doses of 25, 75 or 150 mg/kg/day in mice for 14 consecutive days followed by a single exposure to a 0.5 minimal erythema dose (MED) of solar simulated ultraviolet radiation, produced no cutaneous phototoxicity in animals of these treatment groups and the doses were tolerated with slight mean body weight loss noted for males given ≥75 mg/kg/day and females given 150 mg/kg/day.

b. In vitro phototoxicity study (3T3 fibroblast neutral red uptake assay): [Report WD2005/01434/00]

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Eltrombopag was toxic (IC₅₀ value of 0.543 µg/mL) in the presence of ultraviolet-A (UV-A, wavelength 315 to 400 nm) radiation suggesting phototoxic potential.

To assess the potential relevance of the in vitro phototoxicity finding, a study was performed in the → SKH1-hr hairless mouse. Eltrombopag (batch #TPO-E-07) was given at doses up to 150 mg/kg/day (1607 µg.h/mL, 11-fold the maximum proposed clinical exposure) orally in mice for 14 days followed by a single exposure to 0.5 MED or 10 mJ/cm² of solar-simulated (ultraviolet radiation, UVR) light, there was no evidence of cutaneous phototoxicity at ~1 hour, 4 hours (~Tmax) and 3 days post-UVR exposure. b(4)

Photoclastogenicity was also assessed in two chromosomal aberration assays with CHO cells at cytotoxic concentrations (15 to 29 µg/mL) in the presence of UV light at 700 mJ/cm² (30 MED) [Report WD2006/02097/00 and Report #WD2007/00374/00]. However, there was no evidence of photoclastogenicity was reported at lower light intensity (350 mJ/cm², 15 MED) and at 58.4 µg/ml concentration. The phototoxic effects were observed at high drug concentrations of ≥15 µg/mL and at a light exposure intensity of 30 MED.

Eltrombopag was photoclastogenic in the test. b(5)

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Overall conclusions and recommendations

SB-497115-GR (Eltrombopag) was a selective thrombopoietin receptor agonist and produced proliferation and differentiation of megakaryocytes progenitor cells and released platelets. It showed preferential STAT activity and increase number of platelets specifically only in human and chimpanzee and not in other species. Based on the platelet production property, sponsor proposed to use the compound in patients with thrombocytopenia purpura.

Sponsor demonstrated that the compound had specific species effect of increasing platelets in chimpanzee and humans. Only an insignificant or no effect was seen in other animal species. In the present submission, the studies submitted were on in vitro and in vivo primary and secondary pharmacology, safety pharmacology, in vitro and in vivo ADME studies in dogs and rats, in vitro inhibitory potential on human cytochrome P450 enzymes, acute toxicity study in rats and dogs, 14-day oral toxicity study in mice, rat and dogs, 14-day oral toxicity in female rats with 4 weeks reversibility, 28-week oral study in rats, 7-day oral toxicity study in rabbits and 14-day and 52-week oral capsule study in dogs, 2-week tolerability and TK study in mice, 13-week toxicity in mice, Segment I. reproductive toxicity study in rats, segment II. Reproductive development toxicity studies in rats and rabbits, mutagenicity tests of Ames test, mouse lymphoma mutation assay with L5178Y cells at TK locus with the base and with salt, unscheduled DNA synthesis in rat liver using in vitro/in vitro procedure and, oral micronucleus assay in SD rats. 2-Year oral carcinogenicity studies In mouse and rat and local irritation studies were also submitted.

SB497115 did not show any significant effects on the blood pressure parameters in dogs but it was demonstrated to inhibit the stably transfected hERG-1 cDNA channel (HEK-293 cells) tail current in a concentration dependent manner with IC_{25} , IC_{50} IC_{75} of 0.09, 0.60 and 5.13 μ M (0.04, 0.31 and 2.27 μ g/ml). In dog purkinje fibre assay, SB497115 did not exert any significant effect. At 10 to 25 μ M SB497115 a decrease of the sodium channels currents without an abnormality on EKG and calcium channel was reported.

The ADME studies conducted in mouse, rat, rabbit, dog and monkey have demonstrated that orally administered SB-497115-X (sodium salt) in a solution formulation was absorbed in a linear manner and in greater amount than a capsule formulation. The bioavailability after a 3.52 to 3.6 mg/kg single oral dose of SB-497115-X (sodium salt) as a solution and suspension administration was 83.0 and 91.7% in dogs; 62 and 110% in monkeys. Orally administered compound attained plasma concentration in 3 to 6 hr in monkeys with a low plasma clearance. The half life ($t_{1/2}$) of IV administered compound in dog was 13.9 h and not estimated by oral route and, 7.7 (oral) hr in monkeys. It binds with mouse, rat, dog, monkey and human plasma proteins up to 94% and a minor amount was absorbed in cells. In male and female rats, up to 88 and 90.6% was excreted in feces. In bile duct cannulated rats, the fecal excretion of unchanged compound was 65.7% and 63% in males and females, respectively. A single oral dose 10 mg/kg in intact mice was excreted in feces (72.8% and 76.6% of the dose in males and females). Urinary excretion was \leq 15% of the dose. In

male bile duct-cannulated mice, biliary secretion accounted for a mean of 21.1% of the administered dose and fecal and urinary excretion accounted for about 64.0% and 4.26% of the dose. About 64.8 and 63.7% of the orally administered compound was excreted in feces in male and female rabbits. The urinary excretion was minor, i.e., up to 6.6% in rats and 19.2 % in rabbits. Metabolism was chiefly through conjugation with glucuronic acid in rat, dog and monkeys hepatocyte incubation. SB-497115 was shown to inhibit human cytochrome P450 enzymes and significant inhibition of CYP1A2 and CYP2C9 activity was seen ($IC_{50} = 3.5$ and $9.3 \mu M$, respectively).

Acute toxicity studies in animals were not conducted and sponsor conducted single dose exposure study in rats and maximum tolerated oral dose in dogs. In rat exposure study, the compound attained the peak plasma concentration between 1 to 4 hr and, the plasma concentrations were increased from 1 to 3 times when the compound was mixed in 1% methylcellulose. A single dose of 100 mg/kg was tolerated in dogs excepting emesis, diarrhea, decreased activity and reduction in body weight were reported.

The 2-week toxicity study in mouse was done at the doses of 0, 30, 100 and 300 mg/kg/day by oral gavage (6/sex/group). The dose of 300 mg/kg/day was a toxic and lethal dose and, a dose of 100 mg/kg/day was a well tolerated dose and produced hepatocellular hypertrophy and renal atrophy/degeneration. In another 14-day tolerability studying mouse, 150 and 200 mg/kg/day SB-497115-GR (vehicle HPMC with 0.2% SLS) produced similar plasma concentration in male and female animals and systemic exposure was 1.3 and 1.6folds in males and females at 1.3 times dose.

In 13-week toxicity study in mouse, SB-497119 was administered at 0, 10, 60 or 100 mg/kg/day. In TK group (3/sex/group), the animals were evaluated at week 13. A dose related plasma concentration was seen and, the dose of 100 mg/kg/day was the 'no effect dose'. The target organs of toxicity were not identified.

In 2-week toxicity study in SD rats, SB-497119 was administered at 0, 3, 10 or 40 mg/kg/day. Dose related hepatocellular vacuolation was seen in 40 mg/kg/day treatment group. The NOEL was 10 mg/kg/day. In another study in rats at the doses of 0, 20 and 40 mg/kg/day, produced drug related microscopic changes of hepatocellular vacuolation in 40 mg/kg/day and the dose of 10 mg/kg/day was NOEL.

In 28-week chronic toxicity study in rats, SB-497119 was administered at 0, 3, 10, 30 or 60 mg/kg/day. A dose related plasma concentration was seen in rats and, the doses of 10 and 60 mg/kg/day were 'no effect' and 'lethal' doses. Decreased activity, irregular respiration, pale and cold to touch, increase in absolute and relative reticulocytes were seen in more intensity in males than females included in 60 mg/kg/day treatment group. The target organs of toxicity were liver, blood parameters and kidney. The depletion of lymphoid cells in the spleen, lymph nodes and thymus were seen in 60 mg/kg/day group animals and 9 of 12 males showed thymic atrophy. The dose of 30 mg/kg/day was the highest tolerable dose.

The toxicity of SB-497115 from the doses of 1 to 15 mg/kg/day for 2 weeks was tested in juvenile rats. A treatment related exposure similar in both sexes was observed. Lymph node hemorrhage and myeloid hypercellularity were reported in male and female of 15 mg/kg/day dose. The identified target organs were lymphocytes and bone marrow. In a subsequent juvenile rat study (CD2006/0065/100), SB-497115 was administered at the doses of 0, 5, 15 and 40 mg/kg/day from post partum day 5 to 30, i.e., 28 days duration. One rat of 5 mg/kg/day group died of non treatment related effects. A slight reduction in RBCs, hemoglobin and hematocrit values was seen in 40 mg/kg/day group. The reticulocytes were increased only in 40 mg/kg/day group males. Serum cholesterol (46 and 29% in males and females) and triglycerides (52 and 32% in males and females) were decreased in males of 40 mg/kg/day treatment group.

In 13-day dose ranging study in rabbits, SB-497119 was administered subcutaneously at the dose of 0, 6 or 12 mg/kg/day. A linear dose increase was seen and no treatment related effects were seen in treated animals. In 1-day dose ranging study in rabbits, sc dose of 0, 6 and 12 mg/kg/day was administered. Dose related swelling at the injection site from 6 mg/kg/day doses were seen. The subcutaneous route was considered as unsuitable route of administration in rabbits. The plasma concentrations were 16 and 40.5 and 58.4 ug.h/ml in male and, 18.4, 31.0 and 65.5 ng.h/ml in females and were similar on day 1 and 7 of the study.

In 7-day oral gavage toxicity study in rabbits, SB-497119 at 80, 150 or 200 mg/kg/day produced a linear increase in the plasma concentration. The high dose of the study produced histopathological effect of hepatocellular hypertrophy and erosion of stomach in animals and based on this, the target organ of toxicity was liver in both sexes and, stomach was an additional target organ of toxicity in males of the study. A dose of 150 mg/kg/day was a 'no effect dose (NOAEL)'.

In 14-day toxicity study in dogs, SB-497119 was administered in 4 groups of animals at 0, 3, 10 or 30 mg/kg/day doses and a dose related increase in plasma concentrations was seen in animals. The exposure was about 18.3 and 13.6 folds on day 1 and day 13 in animals of 3 and 30 mg/kg/day treatment groups. The hepatic enzymes and reticulocytes were increased in 30 mg/kg/day treatment group animals and an NOEL was 10 mg/kg/day.

The chronic study of 52-weeks duration in dogs was conducted at 0, 3, 10 or 30 mg/kg/day in gelatin capsules. The exposure in male and female dogs were similar (within 2 folds) within 2 hr of administration of the compound. The AUC values of animals in 30 mg/kg/day were approximately 3, 2 and 3 folds higher on week 4, 13 and 26 than on day 1. Alkaline phosphatase concentrations were increased in a linear manner as 1.7 and 1.9 folds in males and, 1.7 and 2.3 folds of in females included in 10 and 30 mg/kg/day mg/kg/day treatment groups and a dose related hepatocellular vacuolation was seen. Based on this, liver was the target organ of toxicity and an NOEL was 10 mg/kg/day.

Sponsor conducted 104-week mouse carcinogenicity study at the doses recommended by Ex-CAC dated. The study design was appropriate to evaluate the possible tumors formed during the study.

SB-497115-GR (0, 25, 75, 150/115 or 300 mg/kg/day) was given orally to male and female CD-1 mice for up to 104 weeks. The doses of 300 and 150/115 mg/kg/day were lethal and 300 mg/kg/day produced undue mortality the group was terminated. Although a treatment-related decrease in survival in females at all dose levels was seen, yet survival was adequate at week 104 in females given 25 or 75 mg/kg/day to assess carcinogenicity. Administration of SB-497115 was not associated with the induction of tumors. The study was considered as adequate.

The oral gavage 104-week carcinogenicity study in rats was conducted in 4 groups of rats at 0, 10, 20 and 40 mg/kg/day doses. The dose selection was based on CAC-ex recommendations. The sponsors proposed high dose of 40 mg/kg/day for the study was too high and was close to the lethal dose of 60 mg/kg/day, the Committee recommended 5, 15, and 30 mg/kg/day as low, mid and the high doses, respectively. A reduction in the incidences of malignant adenoma in animals (7/22, 2/26 and 1/23 males and, 5/35, 6/22 and 1/17 females) of 0, 25 and 75 mg/kg/day treatment groups was reported but was considered not significant. SB-497115 did not induce tumor of any class, benign or malignant in study rats.

Sponsor conducted Fertility and early embryonic development Study in female rats at oral gavage doses of 0, 10, 20 and 60 mg/kg/day. A decrease in maternal body weight and food consumption was reported in high dose group animals. The embryo- and fetotoxicity and increased pre and post implantation losses were seen in females of the 60 mg/kg/day treatment group. No adverse effects were seen in other low doses of 10 and 20 mg/kg/day. The highest tolerable dose for fertility and reproductive performance in females was identified to be 20 mg/kg/day.

SB-497115 when administered in male rats for 14 days before mating with untreated females till mating confirmed (42 to 46 doses), produced a reduced food consumption related decrease in body weight gain in 40 mg/kg/day group during the initial treatment period of 2 weeks. The mean testes weight of 40 mg/kg/day group males was increased without an effect on mating and fertility of treated males. No effect on growth or external morphology of the fetuses sired by the treated males was seen in rats. Therefore the no-observed adverse effect dose for male fertility was 40 mg/kg/day.

Embryo-fetal developmental toxicity studies were conducted in pregnant rats and rabbits. In rats segment II study, SB-497115-GR was administered at 10, 20 and 60 mg/kg/day from post coitum day 6 to 17. The maternal toxicity of reduced body weight gain and, reduced fetal growth and microscopic reduced skeletal ossification were seen in the fetuses of the 60 mg/kg/day treatment group. There were no treatment-related teratogenic effects in the study.

In rabbit segment II study, SB497115 was administered at the doses of 0, 30, 80 and 150 mg/kg/day from day 7 to 19 postcoitum. The dose of 150 mg/kg/day was lethal and the dose was reduced to 90 mg/kg/day. A minimal adverse effect of reduced stools and body weight retardation was seen in 80 mg/kg/day treatment group animals. No treatment related increased incidence of external, visceral or skeletal malformations or variations were observed. SB-497115-GR was non-teratogenic in the rabbits included in the study and the highest tolerable dose in pregnant rabbits was 80 mg/kg/day.

In a prenatal and post-natal toxicity study in rats, pregnant dams were treated with 10, 20 or 60 mg/kg/day SB-497115 starting from day 6 postcoitum. The high dose of 60 mg/kg/day was toxic and produced body weight loss from day 19 to 21 pc and also severe decrease in food consumption, deaths, decreased activity, bleeding, vaginal bleeding, pale appearance, diarrhea, brown watery feces and ptosis. Two of 4 dams of the group that delivered prior to termination of the group cannibalized their offspring. The F0 females of mid and low dose treatment groups completed the treatment until Day 20 post partum and no adverse effects on pregnancy, parturition or lactation were noted in these groups of animals. In the study, the highest tolerable dose in rats during prenatal and post-natal period can not be identified.

SB-497115 was tested for its mutagenicity in Ames test in 4 strains of *S. typhimurium* (his- to his+) and in *E. coli* (trp- to trp+) in the presence and in the absence of S9 from the concentrations of 62.5 to 2500 ug/plate in triplicate. It was non-mutagenic in the test. The mouse lymphoma TK-/- gene forward mutation assay was performed with free acid, (62.5- 2500 ug/plate), its monoethanolamine (20 to 43 ug/ml) and bismonoethanolamine salt (GR, 0.5 to 14ug/ml) of SB-497115. The compound at the concentration of 1 to 10 ug/ml in the presence and, at 0.5 to 14 ug/ml concentration in the absence of S9 mix was genotoxic in mouse lymphoma L5178Y assay at 24 hr exposure hr and not at 3 hr exposure. SB497115 produced a significant increase in mutation frequencies in L5178Y mouse lymphoma cells following 3 hr exposure in the presence and absence of S9 mix. In oral rat micronucleus test, the micronuclei number was not increased by SB497115. It was not clastogenic in this test. In unscheduled DNA synthesis in rat hepatocytes, groups of rats were treated at 120, 240 or 500 mg/kg twice during 2 to 4 hr and 12 to 14 hr prior to isolating hepatocytes. The compound did not induce unscheduled DNA synthesis in rat hepatocytes.

Sponsor conducted special toxicity studies with the compound to assess its phototoxicity in eyes of female albino CD-1 and pigmented B6C3F1 mice, SB-497115 at 100 mg/kg/day dose induced cataract among B6C3F1 pigmented mice and albino mice and the incidences were increased by UV exposure. The repeated doses of solar-simulated UVR in animals treated at 150 mg/kg/day SB-497115 were lesser than non-exposed animals. B6C3F1 mice given SB-497115 with or without UVR exposure were more sensitive than CD-1 mice. SB-74116 application on the _____ cornea did not affect the mean tissue viabilities and was considered as non-irritant. No tissue histology or analyses of inflammatory mediator levels were done. In rabbit enucleated eye test, the compound solution showed a strong irritant activity but lymph node assay, it did not increase in the incorporation of radioactivity. The compound was a non-sensitizer under the test conditions.

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Sponsor should modify the label as suggested under the labeling section (see Executive Summary) of the present review.

In conclusion, sponsor had demonstrated SB-497115-GR (Eltrombpag) as a selective thrombopoietin receptor agonist which produced proliferation and differentiation of megakaryocytes progenitor cells

Reviewer: Chopra

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and released platelets in chimpanzees and in human platelet preparations. The effect could not be demonstrated in other laboratory animals. The submitted toxicity studies in rat, dog and mice showed that the liver, kidneys, lymph containing tissues and, eyes as target organs of toxicity. Based on the submitted preclinical data of the compound in selective species and the information on the safety and toxicity of the compound, the sponsor provided adequate preclinical data for the approval of compound.

RECOMMENDATIONS:

From preclinical standpoint, the approval of eltrombipag tablet application is recommended.

Signatures (optional):

Reviewer Signature

Yash M. Chopra, M.D., Ph.D.

Supervisor Signature

Adebayo A. Lanionu, Ph.D.

Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

1. CAC-ex memorandum for 104-week mouse and rat carcinogenicity studies.
2. Tables of neoplastic/non-neoplastic incidences in 104-week mouse and rat carcinogenicity studies.

Reviewer: Chopra

NDA No. 22-291

APPENDIX 1

**PHARMACOLOGIST'S REVIEW OF Pre IND 63, 293 (Amendment # 002 SX)
Dated September 29, 2004**

Sponsor & Address: GlaxoSmithKline, King of Prussia, PA 19406

Reviewer: Sushanta Chakder, Ph.D. Pharmacologist, 180

Date of Submission: September 29, 2004

Date of HFD-180 Receipt: September 30, 2004

Date of Review: October 26, 2004

Drug: SB-497115-GR.

Category: Thrombopoietin Receptor (TpoR) Agonist.

Submission Contents: Study protocol for 2-year oral carcinogenicity study in rats.

**Carcinogenicity Assessment Committee (CAC/CAC.EC) Cover Sheet
Review of Carcinogenicity Study Design/Dose Selection~Proposals**

Application (IND/NDA) number: IND 63,293

Division: Gastrointestinal and Coagulation Drug~Eroducts, II- 180

Drug Name: SB.:497115-GR.

Pharmacological Classification: Thrombopoietin Receptor (TpoR) Agonist.

Sponsor/Applicant: GlaxoSmithKline

King of Prussia, PA 19406

Sponsor/Applicant Contact Name: Paula Bursztyn Goldberg, Ph.D.

Sponsor/Applicant telephone/fax number: Tel: 610-787-3722; Fax: 610-787-7062

Date Submitted: September 19, 2004

45-day date: November 14, 2004

Review completion:

Date of CAC review: November 02, 2004

CAC members: Drs. Abby Jacobs, Joe Contrera, John Leighton.

Summary of proposal for review
Species/strain: CD(SD)IGS BR rats.
Number/sex/dose: 60/sex/dose
Route: Oral gavage

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	Male	Female
Doses proposed:	0, 10, 20 and 40 mg/kg/day	0, 10, 20 and 40 mg/kg/day
Basis of dose selection:		
MTD	X	
AUC ratio		
Saturation:		
MFD		

Kinetics submitted:	Rodent	Human
Pharmacokinetics	X	X
Metabolism	X	X
Protein binding	X	X

Notable design features: None.

Summary of Recommendations to CAC

	Male	Female
Doses recommended:	10, 20 and 40 mg/kg/day	10, 20 and 40 mg/kg/day

Basis for recommendation:

The dose selection for the 2-year rat carcinogenicity study is based on toxicity end points (MTD) determined from the results from a 14-day oral toxicity study and a 28-week oral toxicity study with SB-497115-GR in Sprague-Dawley rats. In the 28-week oral toxicity study in rats, SB-497115-GR doses of 0, 3, 10, 30 and 60 mg/kg/day were used. Treatment-related mortalities were observed at an oral dose of 60 mg/kg/day (11 of 12 males and 4 of 12 females). Decreased food consumption and body weight gains (males only), increased liver weights (females only), and microscopic changes in the liver (hepatocellular vacuolation, hypertrophy, centrilobular degeneration and necrosis), kidneys (dilatation, degeneration and regeneration of tubules), adrenal cortex (vacuolation, necrosis of cortex) and pituitary (vacuolation of pars distalis) were observed at this dose. Hematological changes were observed in high dose males (decreased erythrocytes, hemoglobin and hematocrit values and increased platelet levels) and females (decreased hemoglobin and hematocrit values and increased platelet levels). Hepatocellular vacuolation and hypertrophy were also observed in females at 30 mg/kg/day. Thus, it appears that the MTD is in between 30 and 60 mg/kg/day doses.

In the 14-day oral toxicity study in rats, SB-497115-GR doses of 0, 3, 10 and 40 mg/kg/day were used. Males receiving the 40 mg/kg/day dose had decreases in food consumption and suppressed body weight gains (42%). Suppression of body weight gains at the high dose may be related to decreased food consumption. Microscopic changes in the liver (minimal midzonal hepatocellular vacuolation) were observed in males and females receiving the 40 mg/kg/day dose. Thus, 40 mg/kg/day dose was a tolerated dose in this 14-day oral toxicity

study. In a separate study in female rats, it was shown that the hepatocellular changes (midzonal vacuolation) observed at the 40 mg/kg/day dose were reversible during the 4-week recovery period. The plasma exposure levels (AUC values) in male and female rats at the 40 mg/kg/day dose were 672 and 628 $\mu\text{g}\cdot\text{hr}/\text{ml}$, respectively. In humans, an exposure level of 80.9 $\mu\text{g}\cdot\text{hr}/\text{ml}$ was achieved after administration of 10 daily doses of 75 mg/day (1.5 mg/kg/day). The plasma exposure levels in male and female rats at the 40 mg/kg dose are about 8.3 and 7.8 times the human exposure. Based on these findings, doses of 10, 20 and 40 mg/kg/day were selected for the 2-year rat carcinogenicity study, and appear to be appropriate.

The plasma protein binding of SB-497115 was similar in rats and humans (about 99%), and no apparent differences in the metabolite patterns were observed between rats and humans. SB-497115 was genotoxic in the mouse lymphoma cell forward gene mutation (TK⁺) assay.

CAC Concurrence (y/n):

CAC Recommendations:

Comments:

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study. In a separate study in female rats, it was shown that the hepatocellular changes (midzonal vacuolation) observed at the 40 mg/kg/day dose were reversible during the 4-week recovery period. The plasma exposure levels (AUC values) in male and female rats at the 40 mg/kg/day dose were 672 and 628 µg.hr/ml, respectively. In humans, an exposure level of 80.9 µg.hr/ml was achieved after administration of 10 daily doses of 75 mg/day (1.5 mg/kg/day). The plasma exposure levels in male and female rats at the 40 mg/kg dose are about 8.3 and 7.8 times the human exposure. Based on these findings, doses of 10, 20 and 40 mg/kg/day were selected for the 2-year rat carcinogenicity study, and appear to be appropriate.

The plasma protein binding of SB-497115 was similar in rats and humans (about 99%), and no apparent differences in the metabolite patterns were observed between rats and humans. SB-497115 was genotoxic in the mouse lymphoma cell forward gene mutation (TK⁺) assay.

CAC Concurrence (y/n):

CAC Recommendations:

Comments:

Protocol for the 2-year Carcinogenicity Study in Rats:

The sponsor submitted a protocol for the 2-year carcinogenicity study of SB-497115-GR in Sprague-Dawley rats. In the proposed study, SB-497115-GR will be administered by oral gavage to groups of rats (60 animals/sex/group) at 0, 10, 20 and 40 mg/kg/day doses (10 ml/kg). Control animals will be administered the same volume of the vehicle (2% hydroxypropyl methylcellulose with 0.2% sodium lauryl sulfate). Satellite groups consisting of 6 animals/sex/group will be used for toxicokinetic studies. The animals will be observed twice daily for clinical signs. Body weights and food consumptions will be measured once weekly during Weeks 1 through 16 and once every 4 weeks thereafter. At the end of the 104-week dosing period, the surviving animals will be sacrificed and complete necropsies performed. Necropsies will also be conducted of all animals that die or are euthanized in a moribund condition. The following organs/tissues from all animals will be preserved in neutral buffered formalin for histopathological examinations. Histopathologic examinations of all tissues from all groups of animals will be conducted.

adrenal (2)	optic nerve [preserved in Davidson's fixative for all sacrificed animals] (2)
aorta (thoracic)	ovary (2)
brain	pancreas
cecum	pituitary gland
cervix	preputial gland
olfactory gland	prostate
colon	rectum ^a
duodenum	salivary gland (mandibular, parotid, and sublingual)
epididymis (2) ^b	sciatic nerve
esophagus	seminal vesicle (2)
eye [preserved in Davidson's fixative for all sacrificed animals] (2)	skeletal muscle (hind-limb)
femur (femoro-tibial joint)	skin
harderian gland (2)	spinal cord (cervical ^a , thoracic ^a , and lumbar)
heart	spleen
ileum	sternum with bone marrow
injection site(s) (if IV study)	stomach
jejunum	testis (2) ^b
kidney (2)	thymus
larynx	thyroid with parathyroid (2)
lesions	tongue
liver (two lobes)	trachea
lung (2 lobes, preferably right and left, one lobe proximal and one lobe distal, will be examined)	urinary bladder
lymph node (mandibular and mesenteric)	uterus
mammary gland (inguinal)	vagina
nasal cavities and nasopharynx ^a with skull	

^a Collected and held for possible microscopic examination (examination by amendment)
^b Fixed in Bouin's solution.

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For toxicokinetic analysis, samples of blood will be collected from the satellite group animals (6/sex/group) prior to dosing and at 1, 2, 4, 8 and 24 hours after dosing in Weeks 4 and 26.

Proposed Dose Levels: Dose selection for the 2-year rat carcinogenicity study is based on toxicity end points determined from a 14-day oral toxicity study and a 28-week oral toxicity study with SB-497115-GR in Sprague-Dawley rats. In the 28-week oral toxicity study in rats, SB-497115-GR doses of 0, 3, 10, 30 and 60 mg/kg/day were used. Treatment-related mortalities were observed at an oral dose of 60 mg/kg/day (11 of 12 males and 4 of 12 females). Decreased food consumption and body weight gains (males only), increased liver weights (females only), and microscopic changes in the liver (hepatocellular vacuolation, hypertrophy, centrilobular degeneration and necrosis), kidneys (dilatation, degeneration and regeneration of tubules), adrenal cortex (vacuolation, necrosis of cortex) and pituitary (vacuolation of pars distalis) were observed at this dose. Hematological changes were observed in high dose males (decreased erythrocytes, hemoglobin and hematocrit values and increased platelet levels) and females (decreased hemoglobin and hematocrit values and increased platelet levels). Hepatocellular vacuolation and hypertrophy were also observed in females at 30 mg/kg/day. Thus, it appears that the MTD is in between 30 and 60 mg/kg/day doses.

In the 14-day oral toxicity study in rats, SB-497115-GR doses of 0, 3, 10 and 40 mg/kg/day were used. Males receiving the 40 mg/kg/day dose had decreases in food consumption and body weight gains. Microscopic changes in the liver (minimal midzonal hepatocellular vacuolation) were observed in males and females receiving the 40 mg/kg/day dose. Thus, 40 mg/kg/day dose was a tolerated dose in this 14-day oral toxicity study. In a separate study in female rats, it was shown that the hepatocellular changes (midzonal vacuolation) observed at the 40 mg/kg/day dose were reversible during the 4-week recovery period. The plasma exposure levels (AUC values) in male and female rats at the 40 mg/kg/day dose were 672 and 628 µg.hr/ml, respectively. Based on these findings, doses of 10, 20 and 40 mg/kg/day were selected for the 2-year carcinogenicity study in rats.

Fourteen (14)-Day Oral Toxicity Study of SB-497115-GR in Rats (Study #G02012)

Testing Laboratory: GlaxoSmithKline, King of Prussia, PA.
Study Start and Completion Dates: March 07, 2002 and February 19, 2003.

GLP and QAU Compliance Statement: The sponsor included statements of compliance with GLP regulations and Quality Assurance statement.

Animals: - CD@ (SD) IGS BR rats.

Age- approximately 12 weeks old

Body weights- males, 366-442 g; females, 220-275 g.

Drug Lot No.: SB497115-GR, Lot No. F033082, purity 99.3%.

Methods: Four groups of rats (10 animals/sex/group) were administered 0, 3, 10 and 40 mg/kg/day doses of SB497115-GR by oral gavage for up to 15 days (dosing volume, 10 ml/kg). Control animals received the vehicle (2% hydroxypropylmethylcellulose with 0.2% sodium lauryl sulfate). Satellite groups, consisting of 3 animals/sex/group were used for toxicokinetic analysis. The animals were observed daily for mortality and clinical signs. The body weights of the animals were recorded daily, and food consumption was recorded once-weekly. Blood samples for clinical pathology examinations were collected on Day 14 of dosing, and urinalysis was conducted on Day 15 or 16.

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On Days 15 and 16, the main study group animals were sacrificed (5 rats/sex/day), and necropsies performed. The weights of the following organs were recorded: adrenals, brain, epididymides, heart, kidneys, liver, ovaries, seminal vesicles, testes, thymus and prostate.

For histopathologic examinations, the following tissues from all main study group animals were preserved in 10% neutral buffered formalin. Histopathological examinations of all tissues were conducted only of the control and the high dose group animals. Macroscopic abnormalities from the low and mid dose groups were also examined microscopically.

Adrenal glands	Pancreas
* Animal identification	Parathyroids
Aorta (thoracic)	Pituitary
Brain	* Preputial/Clitoral gland
Cecum	Prostate
* Cervix	* Rectum
Colon	* Rib (CCJ)
Duodenum	Salivary glands (mandibular, parotid, sublingual)
Epididymides	Seminal vesicles
Esophagus	Skin
✓ Eyes/Optic nerves	* Skull (base, nose, turbinates, ear canals and accessory tissues)
* Harderian gland	Spinal cord, lumbar
Heart	Spleen
* Hind limb, right	Sternum (includes skeletal muscle and bone marrow)
Hock, right	Stomach
Ileum	Testes
Jejunum	Thymus
Kidneys	Thyroid
* Larynx	Tongue
Liver (left lateral lobe and median lobe)	* Trachea
Lung	Urinary bladder
Lymph nodes (mandibular, mesenteric)	Uterus
Macroscopic observations	Vagina
Mammary gland	
Ovaries	

* Tissue was not processed or examined microscopically.
✓ Eyes and optic nerves were fixed in Davidson's fixative for 24-72 hours, transferred to 70% alcohol for a minimum of 24 hours, trimmed and processed. Residual unprocessed tissue was stored in 10% neutral buffered formalin.
✓ Testes were fixed in Bouin's fixative for approximately 18-72 hours, transferred to 70% alcohol, trimmed and processed prior to storage in 10% neutral buffered formalin.

For toxicokinetic analysis, samples of blood were collected prior to dosing and at 1, 2, 4, 8, 12 and 24 hours after dosing on Days 1 and 14 (3 animals/sex/sampling time). Plasma drug concentrations were measured by LC/MS/MS analysis using negative-ion Turbo IonSpray ionization.

Results:

Clinical signs: No treatment related clinical signs were observed in any group.

Mortality: There were no deaths of animals in any group.

Body Weight: The initial mean body weights of the male and female animals were 406.5±6.7 g and 252.9±4.4 g, respectively. The body weight gain of males receiving the high dose was suppressed as compared with that of controls (58.3% of control body weight gains). Control group females had no gain in the body weight during the 15-day dosing period. Females receiving the low dose had increased body weight gains. The mean body weights and body weight gains of different groups of rats are shown in the Table below.

Parameter	Control		3 mg/kg/day		10 mg/kg/day		40 mg/kg/day	
	M	F	M	F	M	F	M	F
Body Wt. (g) on Day 0	406.5	252.9	405.7	250.9	404.9	252.2	406.4	252.3
Body Wt. (g) on Day 15	435.5	252.9	437.7	271.3	433.7	255.2	423.3	252.6
Body Wt. gain (g)	29.0	0.0	32.0	20.4	28.8	3.0	16.9	0.3
Body Wt. gain (% control)	100%		110.3%		99.3%		58.3%	

Food Consumption: Mean food consumptions of the control male and female animals before initiation of dosing were 31 and 24 g/animal/day, respectively. Males receiving the 40 mg/kg/day dose had a slight decrease in food consumption during the first week of dosing (10%).

Hematology: Males receiving the high dose had slightly higher reticulocyte levels (15.8%), and treatment group males had slightly higher platelet levels (11.3% to 14.4%, not dose related).

Clinical Chemistry: No significant changes in the clinical chemistry parameters were observed in any group.

Urinalysis: Increased urine protein concentrations (≥ 1 g/L) were observed in males at 10 mg/kg/day (4 of 10) and 40 mg/kg/day (7 of 10) doses. These changes were generally associated with reduced urine volume and/or increased urine specific gravity. No microscopic changes in the kidney were observed in these animals.

Gross Pathology: No treatment related gross pathological changes were observed in any group.

Organ Weights: The absolute and relative (to body weight) weights of the testes of males were higher than that of controls (approximately 11% to 15%).

Histopathology: Treatment related microscopic changes in the liver, characterized as midzonal hepatocellular vacuolation, were observed in males and females receiving the 40 mg/kg/day dose. Midzonal hepatocellular vacuolation was observed in 1/10, 0/10, 0/10 and 3/10 male rats, and 1/10, 0/10, 0/10 and 10/10 female rats at 0, 3, 10 and 40 mg/kg/day doses, respectively.

Toxicokinetics: Maximum plasma concentrations (C_{max}) and $AUC_{(0-24)}$ of SB-497115 increased with increasing doses in both male and female rats. No differences in C_{max} and $AUC_{(0-24)}$ were observed between males and females. The C_{max} was reached in 1.0 hour at 3 and 10 mg/kg/day doses and in 2.0 hours at 40 mg/kg/day. Plasma toxicokinetic parameters of SB-497115 in male and female rats are shown in the Table below.

Mean (S.D.) pharmacokinetic parameters for SB-497115 are given below:

Dose (mg/kg/day)	Sex (n=3)	C _{max} (ng/mL)		AUC(0-24) (ng.h/mL)		T _{max} * (hours)	
		Day 1	Day 14	Day 1	Day 14	Day 1	Day 14
3	M	2.49 (1.22)	5.01 (1.56)	13.5 (1.6)	25.2 (3.6)	1.02 (1.00-1.00)	1.00 (1.00-1.00)
10	M	22.6 (8.1)	32.1 (7.0)	107 (26)	198 (37)	1.00 (1.00-1.00)	1.02 (1.00-1.02)
40	M	76.2 (11.8)	71.6 (14.4)	613 (105)	672 (27)	1.00 (2.00-1.00)	1.02 (1.00-1.02)
3	F	3.56 (1.77)	8.01 (3.54)	14.0 (6.7)	28.6 (17.1)	1.00 (1.00-1.00)	1.02 (1.02-1.03)
10	F	19.7 (13.7)	30.5 (3.7)	97.4 (24)	130 (25)	1.00 (1.00-1.07)	1.02 (1.00-1.02)
40	F	79.1 (16.0)	88.2 (3.7)	611 (115)	628 (114)	1.00 (2.00-2.05)	1.01 (2.00-1.03)

* T_{max} expressed as median (range)

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In the 2-week oral toxicity study with SB-497115 in Sprague-Dawley rats, groups of animals were administered 0, 3, 10 and 40 mg/kg/day doses of the drug. There was no mortality in any group. The body weight gains of the high dose male rats were lower than that of controls. Treatment related microscopic changes in the liver (midzonal vacuolation) were observed in male and female animals at 40 mg/kg/day. The 10 mg/kg/day dose was the no effect dose in this 2-week toxicity study. The 40 mg/kg/day dose was a tolerated dose in this study.

28-Week Oral Toxicity Study of SB-497115-GR in Rats (Study #G03114)

Testing Laboratory:

Study Start and Completion Dates: June 16, 2003 and September 17, 2004.

GLP and QAU Compliance Statement: The sponsor included statements of compliance with GLP regulations and Quality Assurance statement.

Animals: — CD@ (SD) IGS BR rats.

Age- approximately 12 weeks old,

Body weights- males, 331-383 g; females, 215-243 g.

Drug Lot No.: SB497115-GR (bis-monoethanolamine salt of SB-497115), Lot No. F033082, purity 99.3%.

Methods: Four groups of rats (12 animals/sex/group) were administered 0, 3, 10, 30 and 60 mg/kg/day doses of SB497115-GR by oral gavage for 28 weeks (dosing volume, 10 ml/kg). Control animals received the vehicle (2% hydroxypropylmethylcellulose with 0.2% sodium lauryl sulfate). The animals were observed twice daily for mortality and clinical signs. The body weights of the animals were recorded pre-dose, on the first day of dosing, and once a week thereafter. Food consumption was recorded daily. Blood samples for clinical pathology examinations were collected during Week 4, and Week 28 (prior to sacrifice), and urine samples were collected before blood collection.

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In Week 28, the surviving animals were sacrificed, and complete necropsies performed. The weights of the following organs were recorded: adrenals, brain, heart, kidneys, liver, ovaries, prostate, lung, spleen, testes and thymus.

For histopathologic examinations, the following tissues from all main study group animals were preserved in 10% neutral buffered formalin. Eyes and optic nerves were preserved in Davidson's fixative. Histopathological examinations of all tissues were conducted only of the control and 30 and 60 mg/kg/day dose-group animals. Macroscopic abnormalities, and the suspected organs (liver, pituitary, adrenal, testes, femur/tibia) from the low and mid dose groups were also examined microscopically.

Tissues Fixed	Tissues Examined	Tissues Fixed	Tissues Examined
Adipose	X	Ovaries	X
Animal identification tags		Pancreas	X
Aorta (thoracic)	X	Parathyroids	X
Brain	X	Pituitary	X
Cecum	X	Preputial gland	X
Cervix	X	Prostate	X
Colon gland		Rectum	X
Colon	X	Salivary gland	X
Diaphragm	X	submandibular	X
Epiglottides	X	sublingual	X
Esophagus	X	sublingual	X
Eyes/Optic Nerves	X	testis	X
Femur (Femoral (All joints))	X	Skeletal muscle (hind limb)	X
Harderian gland		Skin	X
Heart	X	Spinal cord	X
Jejunum	X	ovary	X
Kidneys	X	thoracic	X
Larynx	X	lumbar	X
Lepidoptera	X	Spleen	X
Liver (two lobes)	X	Stomach with bone marrow	X
Lung	X	Stomach	X
Lymph node		Testes	X
mandibular	X	Thymus	X
mesenteric	X	Thyroids	X
Mammary gland (duodenal)	X	Tongue	X
Nasal turbinate		Trachea	X
Nasopharynx		Urinary bladder	X
Optic nerves	X	Uterus with cervix	X
		Vagina	X

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For toxicokinetic analysis, samples of blood were collected prior to dosing and at 1, 2, 4, 8, 12 and 24 hours after dosing in Week 18 (3 animals/sex/sampling time). Plasma drug concentrations were measured by LC/MS/MS analysis using negative-ion Turbo IonSpray ionization.

Results:

Clinical signs: Hunched posture, red nasal discharge and yellow hair coat in the perianal area were observed in males and females receiving the high dose.

Mortality: There were treatment related mortalities at the 60 mg/kg/day dose. Eleven (11) of 12 males and 4 of 12 females from this group died or were sacrificed moribund (in Weeks 2-16 and 26-28) prior to scheduled termination. One male from the 30 mg/kg/day group was sacrificed (had a Zymbal's gland carcinoma), and another male from this group was found dead. One male from the 10 mg/kg/day group, and three control females were found dead during the dosing period. The causes of deaths of these animals were not known.

Body Weight: The initial mean body weights of the male and female animals were 369±9.4 g and 235±6.3 g, respectively. At Week 28, the mean body weights of the surviving high dose males were lower than those of controls. The body weight gains of the surviving high dose males were also lower than that of controls in Week 13 (80.5% of control).

Percent change in mean body weights and body weight gains of different groups of rats are shown in the Table below.

Parameter	Control		3 mg/kg/day		10 mg/kg/day		30 mg/kg/day		60 mg/kg/day	
	M	F	M	F	M	F	M	F	M	F
Body Wt. (g) on Day -1	369	235	367	234	368	235	368	233	368	233
Body Wt. (g) in Week-13	405	244	404	254	414	256	413	252	397	247
Body Wt. gain (g)	36	9	37	20	46	21	45	19	29	14
Body wt. gain (% control)	100%	100%	102.8%	222.2%	127.8%	233.3%	125%	211.1%	80.5%	155.6%
Body Wt. (g) in Week 28	433	261	425	265	433	267	439	267	390	260
Body Wt. change (%)	0	0	-1.85%	1.53%	0%	2.3%	2.3%	2.3%	-9.9%	-0.4%

Food Consumption: It was stated that the food consumptions of the high dose males and females were lower than that of controls (data was provided in terms of pellet counts).

Hematology: Males receiving the high dose had decreased erythrocyte counts (13.3%), lower hemoglobin (18%) and hematocrit values (16%), increased platelet counts in Week 4 (28.4%) and increased reticulocyte counts (188%) in Week 4. High dose females had slightly lower hemoglobin, and hematocrit values (10% both) and increased platelet counts (10%).

Clinical Chemistry: Males receiving the high dose had slightly higher alkaline phosphatase (26%) and AST levels (21%) and decreased total cholesterol levels (47%).

Urinalysis: Increased urine protein concentrations (about 1.8-fold) and protein/creatinine ratios were observed in males at 30 and 60 mg/kg/day in Week 4, and at 30 mg/kg/day in Week 28.

Gross Pathology: Males receiving the high dose had pale tan foci in the liver (6/12), stomachs/intestines distended with orange material (6/12), and pale eyes.

Organ Weights: The tissue/organ weights of the high dose males were not available, as all animals were sacrificed or died prior to scheduled sacrifice. The absolute (17.9%) and relative (to body weight; 21%) liver weights of the high dose females were higher than that of controls.

Histopathology: Treatment related microscopic changes were observed in the liver, adrenal cortex and pituitary of high dose males and females. Microscopic changes in the liver included vacuoles in the hepatocytes in the periportal and midzonal regions, centrilobular degeneration and necrosis, were observed in both males and females. Changes in the kidneys were also observed in high dose males. Vacuolation and necrosis of the adrenal cortex (zona fasciculata) were observed in 3 (of 12) males and 1 (of 12) female receiving the high dose. Inflammation (minimal; 4/12) and degeneration (minimal to mild; 9/12) of the heart were observed in males receiving the high dose. One male receiving the 60 mg/kg/day dose had vacuolated cells in the pars distalis of the pituitary. Endosteal hyperostosis in the femur was observed in 2 males and in the tibia was observed in 1 female receiving the high dose. Depletion of lymphoid cells in the spleen, lymph nodes and the thymus (including lymphocyte necrosis)

were observed in males and females at 60 mg/kg/day. Histopathological changes observed in male and female rats are shown in the Table below.

Organs/Incidences	MALES					FEMALES				
	Control	3	10	30	60	Control	3	10	30	60
Adrenal -vacuolation and necrosis, cortex	0	0	0	0	3	0	0	0	0	1
Pituitary -vacuoles, Pars distalis	0	0	0	0	1	0	0	0	0	0
Liver -degeneration, necrosis, centrilobular -vacuoles, hepatocytes -hypertrophy, hepatocytes	0	0	0	0	9	0	0	0	0	1
Kidney -glomerulopathy -tubule, dilatation -Cyst -degeneration, regeneration, tubules -hyperplasia, urothelium, renal pelvis	4	0	0	1	7	0	0	0	0	0
Bone, Femur -hyperostosis, endosteal	0	0	0	0	2	0	0	0	0	0
Head, coronal -Zymbal's gland carcinoma	0	0	0	0	1	0	0	0	0	0

Toxicokinetics: Maximum plasma concentrations (C_{max}) and $AUC_{(0-4)}$ of SB-497115 increased with increasing doses in both male and female rats. No apparent differences in C_{max} and $AUC_{(0-4)}$ were observed between males and females. The T_{max} ranged for 1.0 hr (at 3 mg/kg) to 4.0 hrs (at 60 mg/kg). Plasma toxicokinetic parameters of SB-497115 in male and female rats are summarized in the Table below.

A summary of the toxicokinetics parameters for SB-497115 is given below:

Sex	Dose (mg/kg/day)	Week	$AUC_{(0-4)}$ (ng·h/mL)	C_{max} (ng/mL)
Male	3	28	21130.0	4478.3
	10	28	83683.4	10444.9
	30	28	650633.0	66496.3
	60	28	1743661.7	64003.3
Female	3	28	28523.1	6233.4
	10	28	129040.3	21518.1
	30	28	671090.9	83434.1
	60	28	1049633.1	148996.6

a. For males given 60 mg/kg/day, AUC was calculated from 1 to 4 hours after dosing.

In the 28-week oral toxicity study with SB-497115 in Sprague-Dawley rats, groups of animals were administered 0, 3, 10, 30 and 60 mg/kg/day doses of the drug. Treatment related mortalities were observed at the 60 mg/kg/day dose. Decreased body weight gain and food consumption, microscopic changes in the liver (hepatocellular vacuolation, hypertrophy, degeneration and necrosis), kidney (dilatation, degeneration and regeneration of tubules), adrenal cortex (vacuolation/degeneration) and

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pituitary (vacuolation) were observed at 60 mg/kg/day. Decreased red cell counts and increased reticulocyte counts were also observed at this dose. Thus, it appears that the MTD is between 30 and 60 mg/kg/day in this 28-week toxicity study.

Metabolism of SB-497115 in Rats (Study # D102031).

The study was conducted to identify and quantify the major metabolites of SB-497115 in plasma, liver, bile, urine and feces following oral administration of a single 10 mg/kg dose of [¹⁴C]-SB-497115-GR to intact and bile duct cannulated rats. Profiles of radioactive drug-related material were quantified by HPLC with radiochemical detection. Structural characterization of metabolites was carried out on selected samples by mass spectrometry (HPLC-MS, HPLC/MS-MS and LC-NMR).

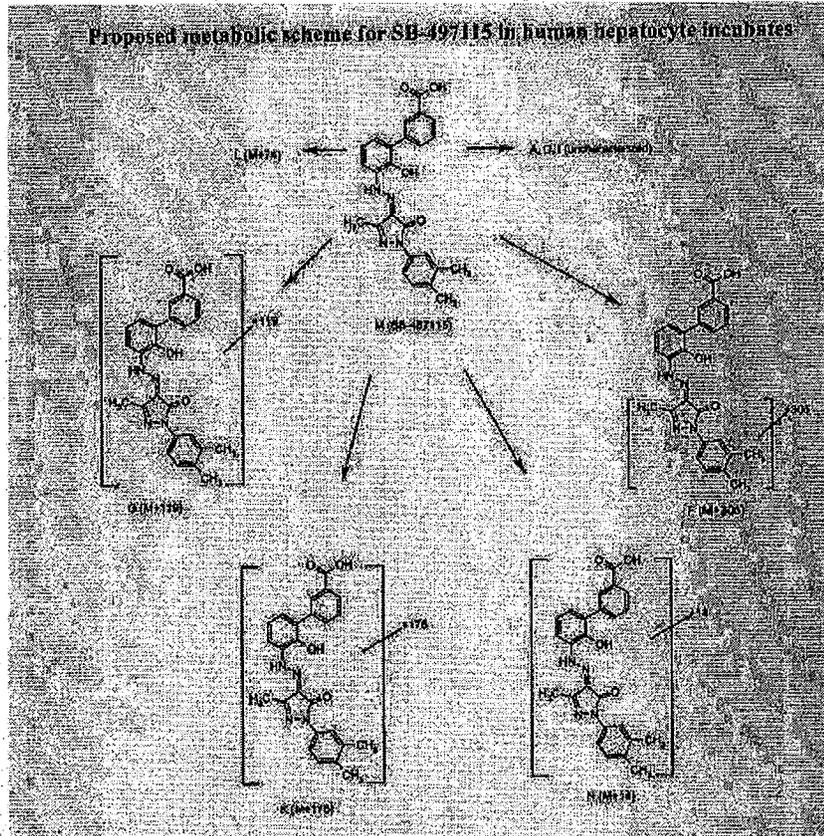
Following a single oral administration of [¹⁴C]-SB-497115-GR to male and female rats, the principal radiolabeled component in plasma at all time points (4, 24 and 48 hours) was unchanged SB-497115 (89% and 76% at 4 hr, and 42% and 58% at 24 hr, in males and females, respectively). Another notable radioactive component was metabolite K/O (co-eluted) which is a product of glucuronidation/oxidation. This metabolite consisted of 33% and 22% of the plasma radioactivity at 24 hr in males and females, respectively. At 48 hrs after dosing, the unchanged SB-497115 was still the major component in the plasma of both male (59%) and female (78%) rats. In addition, metabolites, K/O and AD accounted for approximately 16% and 10% of plasma radioactivity respectively. Liver metabolite patterns were similar to that of plasma. Intact drug was the major radioactive component in the liver at all time points. In addition K/O and AD accounted for about 5%-27% of the radioactivity.

In bile duct-cannulated rats, the drug was eliminated both as an intact drug and metabolites. The major route of metabolism was glucuronidation and glutathione conjugation. Oxidation was a minor route of metabolism. In intact rats, the elimination of drug-related material was mainly via feces (88-91%). The principal radioactive component was the intact drug, and two minor pathways included glucuronidation and oxidation. Urinary excretion played a minor role (5-7%), and unchanged drug was not detected in any of the urine samples.

A preliminary *in vitro* investigation of the metabolism of [¹⁴C]SB-497115 in the rat, dog, cynomolgus monkey and man (Study #D100903).

[¹⁴C]SB-497115 was incubated at concentrations of 10 and 50 μM with hepatocytes prepared from rat, dog, monkey and human livers for 0, 6 and 24 hours. Extracts of the incubates were analyzed by radio-HPLC and selectively by LC/MS in order to compare the metabolism of SB-497115 across species.

Metabolism of SB-497115 was studied with hepatocytes from four human livers. Except for one human liver, the metabolism of the compound by the human hepatocyte samples was low. The major metabolic route observed was conjugation with either cysteine or glucuronide. Conjugation with glutathione and oxidation leading to formation of M+14 metabolite (possible carbonyl formation) were also observed as minor metabolic pathways in the human hepatocytes. The putative metabolic pathways of SB-497115 in human hepatocytes are shown in the Figure below.



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In hepatocytes isolated from animals, all metabolic routes observed in human hepatocytes were identified. Conjugation with cysteine or glucuronide were the predominant pathways in all species, including humans. The minor pathways included formation of a glutathione conjugate, formation of the M+14 metabolite and a possible carboxylic acid. A few other minor metabolites, not observed in humans, were also detected in nonclinical species. No human specific metabolites were detected.

Preliminary investigation of protein binding of SB-497115 to rat, dog, monkey and human plasma protein and to determine the bound to plasma ratio of SB-497115 in these species.

The plasma protein binding of SB-497115 in rat, dog, monkey and human plasma was determined *in vitro* by equilibrium dialysis at final SB-497115 concentrations of 2000 and 6000 ng/ml. Blood partitioning of SB-497115 in rat, dog, monkey and human blood was determined at nominal blood concentrations of 2000 and 6000 ng/ml.

The plasma protein binding of SB-497115 in the plasma of rats, dogs, monkeys and humans was very high ($\geq 99\%$) and similar at both 2000 and 6000 ng/ml concentrations. The plasma protein bindings of SB-497115 in the rat, dog, monkey and human plasma are shown in the Table below.

Species	Target Plasma Concentration (ng/mL)	Fraction Bound to Plasma Protein ^a (%)
Rat	2000	>99.4
	6000	>99.3
Dog	2000	>99.3
	6000	>99.3
Monkey	2000	>99.0
	6000	>99.7
Human	2000	>99.4
	6000	>99.8

^a Recoveries in the protein binding experiments were highly variable, ranging from 56.7 to 197.7 percent.

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Moderate partitioning of SB-497115 in the blood cells was observed in all species.

Genotoxicity:

The genotoxic potential of SB-497115 (free acid) was examined in the bacterial reverse mutation assay (Ames test) and the mouse lymphoma cell (L5178Y) forward gene mutation (TK⁺) assay. SB-497115 was negative in the Ames test. In the mouse lymphoma cell TK assay, it induced statistically significant increases in mutation in the presence and absence of metabolic activation (both small and large colonies were induced).

SB-497115-GR, the ethanolamine salt of SB-497115 was tested for its potential genotoxicity using the mouse lymphoma cell (L5178Y) forward gene mutation (TK⁺) assay. SB-497115-GR was genotoxic in the mouse lymphoma TK assay in the absence of metabolic activation following 24-hour treatment period.

SB-497115-GR was also tested for its genotoxic potential in the *in vivo* rat bone marrow micronucleus assay and the rat liver unscheduled DNA synthesis (UDS) assay. SB-497115-GR was not clastogenic in the rat bone marrow micronucleus assay after 2 oral administrations of 120, 240 and 500 mg/kg doses. It was not genotoxic in the rat liver UDS assay at 120, 240 and 500 mg/kg oral doses.

The 2-aminoethanol salt of SB-497115, was tested for potential genotoxicity in the mouse lymphoma cell (L5178Y) forward gene mutation (TK⁺) assay. 2-aminoethanol was genotoxic at the TK locus of mouse lymphoma L5178Y cells in the absence of S9-mix following 24 hours treatment period. It was not genotoxic in the presence of metabolic activation.

Reviewer: Chopra

NDA No. 22-291

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Sushanta Chakder, Ph.D.

Date

Comments:

Jasti B. Choudary, B.V.Sc., Ph.D

Date

cc:
IND
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Chakder

R/D Init.: J. Choudary 10/25/04

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/s/

Sushanta Chakder
10/26/04 11:44:10 AM
PHARMACOLOGIST

Jasti Choudary
10/26/04 03:15:19 PM
PHARMACOLOGIST

Reviewer: Chopra

NDA No. 22-291

**PHARMACOLOGIST'S REVIEW OF IND 63,293
(Amendment # 001SX dated September 29, 2004)**

Sponsor & Address: GlaxoSmithKline
King of Prussia, PA

Reviewer: Tamal K. Chakraborti, Ph.D.
Pharmacologist, HFD-180

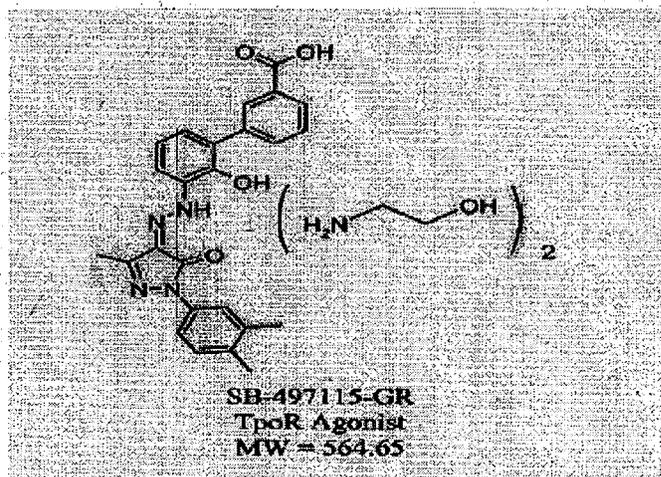
Date of Submission: September 29, 2004

Date of Receipt: September 30, 2004

Date of Review: October 26, 2004

Drug: SB-497115-GR (*bis*-monoethanolamine salt of SB-497115)

Structure:



Category: Thrombopoietin receptor (TpoR) agonist

Indication: SB-497115-GR is indicated for ——— treatment of thrombocytopenia associated

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IND 63,293 Amendment 001SX

Submission Contents:

1. Dose selection/protocol for 2-year oral (gavage) carcinogenicity study in CD-1 mice.
2. 14-Day oral toxicity study in mice (CD2003/00476/00).
3. 2-Week oral gavage tolerability and toxicokinetic (TK) study in CD-1 mice (CD2004/00836/00).
4. 13-Week oral toxicity study in mice (CD2004/00627/00)
5. Genotoxicity studies
6. Protein binding studies

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Reviewer: Chopra

NDA No. 22-291

IND 63,293 Amendment 001SX

**Carcinogenicity Assessment Committee (CAC/CAC-EC) Cover Sheet
Review of Mouse Carcinogenicity Study Design/Dose Selection Protocols**

Application (IND) Number: 63,293

Division: Gastrointestinal and Coagulation Drug Products

CAS Registration Number: 496775-62-3

Drug Name: SB-497115-GR

Pharmacological Classification: Thrombopoietin receptor (TpoR) agonist

Sponsor/Applicant: GlaxoSmithKline (GSK).

Sponsor/Applicant Contact Name: Paula Bursztyrn Goldberg, Ph.D.

Sponsor/Applicant Telephone and Fax Number: Tel. (610) 787-3722; Fax: (610) 787-7062

Date of Receipt (Stamp Date): September 30, 2004

45-Day Date (From Submission Stamp Date): November 14, 2004

P/T Reviewer: Tamal K. Chakraborti, Ph.D.

Date Review Completed:

Date of CAC review: November 2, 2004

CAC Members: Abby Jacobs, Ph.D., HFD-024, Acting Chair
Joseph Contrera, Ph.D., HFD-901, Member
John Leighton, Ph.D., HFD-150, Member
Jasti Choudary, B.V.Sc., Ph.D., HFD-180, Supervisory Pharmacologist

IND 63,293 Amendment 001SX

Summary of Proposal for Review:

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Species/Strain: Mice - CD-1®(ICR)BR

Number/Sex/Dose: 60/sex/dose

Route: Oral gavage

	<u>Male</u>	<u>Female</u>
Doses Proposed:	0, 30, 100, 200 mg/kg/day	0, 30, 100, 200 mg/kg/day

Basis of Dose Selection:

MTD	X	X
AUC Ratio	_____	_____
Saturation	_____	_____
MFD	_____	_____
PD	_____	_____
Other	_____	_____

Kinetics Submitted:	<u>Rodent</u>	<u>Human</u>
Pharmacokinetics	X	X
Metabolism	_____	_____
Protein Binding	X	X

Genotoxicity: Both SB-497115 (free acid) and SB-497115-GR (ethanolamine salt form of SB-497115) were found to be positive in L5178Y TK⁺ mouse lymphoma assay. SB-497115 was found to be negative in Ames assay. SB-497115-GR was negative in unscheduled DNA synthesis (UDS) assay using *ex vivo* rat hepatocytes and *in vivo* rat bone marrow micronucleus assay using oral route.

Notable Design Features: None

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Summary of Recommendations to CAC

Doses Recommended by Reviewer: Male: 0, 30, 100, 200 mg/kg/day
Female: 0, 30, 100, 200 mg/kg/day

Basis for Recommendation:

SB-497115-GR is the *bis*-monoethanolamine (MEA) salt form of SB-497115, a non-peptide, orally active thrombopoietin receptor (TpoR) agonist in development for treatment of thrombocytopenia associated with immune thrombocytopenia purpura (ITP).
The maximum recommended human therapeutic dose for SB-497115-GR has not been determined yet, however, dose-dependent increases in platelet counts (1.3- to 1.5-fold) have been observed in normal male subjects following 10 daily doses of 30 to 75 mg. The mean AUC_{0-24h} of 80.9 µg.h/ml was achieved after 75 mg dose.

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In this submission, the sponsor has submitted the rationale or the basis for the dose selection and the protocol for the proposed 2-year oral carcinogenicity study in CD-1 mice at 30, 100, 200 mg/kg/day doses and the reports of 2-week oral toxicity study (Study No. CD2003/00476/00, Non-GLP) in CD-1 mice, 2-week oral tolerability and toxicokinetic study (Study No. CD2004/00836/00, Non-GLP) in CD-1 mice and 13-week oral toxicity study (Study No. CD2004/00627/00) in CD-1 mice. In addition, the sponsor also submitted reports of genotoxicity [L5178Y TK⁺ mouse lymphoma assay with SB-497115 and SB-497115-GR, Ames assay with SB-497115, rat *in vivo* oral micronucleus test with SB-497115-GR and unscheduled DNA synthesis (UDS) assay with SB-497115-GR using *ex vivo* rat hepatocytes] and protein binding studies with SB-497115.

The sponsor has proposed to conduct a 2-year oral carcinogenicity study in CD-1 mice with SB-497115-GR at 30, 100, and 200 mg/kg/day doses. The sponsor's dose selection was based on the toxicity endpoints determined from results of the 2-week oral toxicity study (CD2003/00476/00) in CD-1 mice, 2-week oral tolerability and toxicokinetic study (CD2004/00836/00) in CD-1 mice and 13-week oral toxicity study (CD2004/00627/00) in CD-1 mice.

In a 14-Day oral (gavage) toxicity study (CD2003/00476/00) in the CD-1 mouse, animals were administered SB-497115-GR at 0, 30, 100 and 300 mg/kg/day. The vehicle control group received 2% hydroxypropylmethylcellulose (HPMC) with 0.2% sodium lauryl sulfate. Treatment-related mortality was observed at 300 mg/kg/day in both sexes and all mice at this dose level were sacrificed on Day 10 due to deteriorating clinical conditions. The NOAEL appeared to be 30 mg/kg/day in both sexes. Histopathological changes were observed in the following organs at 300 mg/kg/day in both sexes: liver (centrilobular hypertrophy, sinusoidal pigment deposit, coagulative hepatocellular necrosis, hepatocellular vacuolation in females, peribiliary inflammation), kidney (tubular vacuolation, necrosis/regeneration of cortical medullary tubule), stomach (non-glandular ulcer) stomach (non-glandular ulcer), and lung (Clara cell hypertrophy and arterial smooth muscle vacuolation). Histopathological changes were also observed predominantly in males at 100 mg/kg/day in the following organs: liver (hepatocellular hypertrophy in 3 of 6 males, coagulative hepatocellular necrosis in 3 of 6 males), kidney (necrosis/regeneration of cortical medullary tubule in 1 of 6 males and in 1 of 6 females). The

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target organ of toxicity appeared to be the liver, kidney, lung and stomach. It is to be mentioned here that the sponsor did not state whether the necrosis in the liver was single cell or focal necrosis.

In a subsequent 2-week oral tolerability and toxicokinetic (TK) study (CD2004/00836/00) in the CD-1 mouse, animals were tested at 150 and 200 mg/kg/day of SB-497115-GR. This is an exploratory study and the sponsor did not conduct any histopathological examinations of any organ or tissue. There was no mortality and doses of 150 and 200 mg/kg/day were well tolerated. Exposure was found to be dose-proportional with a mean AUC_{0-24h} of 1358 µg.h/ml at 200 mg/kg/day.

In a 13-Week oral (gavage) toxicity study (CD2004/00627/00) in the CD-1 mouse, animals were administered SB-794115-GR at 0, 10, 60 and 100 mg/kg/day. The NOAEL appeared to be 100 mg/kg/day in both sexes. The target organ of toxicity could not be identified in the absence of any organ toxicity at any of the tested doses. The dose selection does not appear to be appropriate, as the highest tested dose did not produce any signs of toxicity. The 200 mg/kg/day should have been included in this study for the following reasons: 1) 200 mg/kg/day dose in a previous tolerability and TK study (CD2004/00836/00) did not cause any mortality and 2) lack of histopathology data at 200 mg/kg/day dose. It is worth mentioning here that histopathological changes (hepatocellular hypertrophy, coagulative hepatocellular necrosis, necrosis of cortical medullary tubule in the kidney in males) observed at 100 mg/kg/day in a previous 14-day toxicity study (CD2003/00476/00) were absent in the 13-week study. The apparent reason for the absence of liver and kidney findings at 100 mg/kg/day animals in the 13-week study is not clear. However, based on the absence of any mortality at 200 mg/kg/day in a previous tolerability study and lack of mortality and histopathology findings at 100 mg/kg/day in this 13-week study, it appears that the maximum tolerated dose is greater than 100 mg/kg/day and less than 300 mg/kg/day.

The sponsor did not provide any *in vitro* metabolism data with SB-497115. An *in vivo* metabolism study in the mouse is in progress. The *in vitro* binding of SB-497115 to plasma proteins was determined using equilibrium dialysis at concentrations of 2000 and 6000 ng/ml in rat, dog, monkey and human plasma. Mean protein binding was >99% in human plasma and 94% in mouse plasma.

The genotoxic potential of SB-497115-GR (bis-monoethanolamine salt form of SB-497115) was evaluated *in vitro* (L5178Y TK⁺ mouse lymphoma assay), *in vivo* (rat oral micronucleus assay) and *ex vivo* (unscheduled DNA synthesis using rat hepatocytes). In addition, Ames assay and L5178Y TK⁺ mouse lymphoma assays were also conducted with SB-497115 (free acid). Both SB-497115 (free acid) and SB-497115-GR (ethanolamine salt) were found to be positive in L5178Y TK⁺ mouse lymphoma assay. In this mouse lymphoma assay, both small and large colonies were increased in the presence and in the absence of S9 compared to control. SB-497115 was found to be negative in the Ames assay, unscheduled DNA synthesis (UDS) assay using *ex vivo* rat hepatocytes and *in vivo* rat bone marrow micronucleus assay using oral route. Based on the positive results in the mouse lymphoma assay, SB-497115 appears to have genotoxic potential.

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It is to be mentioned here that using human exposure of 80.9 µg.h/ml (mean AUC_{0-24h} in normal male subjects given 10 daily doses of 75 mg SB-497115-GR/day) anticipated exposure margins at 30, 100 and 200 mg/kg/day would be approximately 3.7-, 8- and 17-fold, respectively. However, the sponsor did not mention whether the exposure (AUC) was for the unbound drug in the plasma. The sponsor also did not measure exposure levels (AUCs) for metabolites. In addition, metabolic profiles of SB-497115-GR in humans and mice are also unknown.

In summary, 200 mg/kg/day appears to be an acceptable high dose for the proposed carcinogenicity study in CD-1 mice. The sponsor's proposed high dose of 200 mg/kg/day appears to be appropriate for the following reasons: 1) mortality observed at 300 mg/kg/day in a 14-day toxicity study and 2) absence of any toxicity at 100 mg/kg/day in the 13-week study. Based on this information, it appears that the sponsor's proposed high dose of 200 mg/kg/day (approximately 17-fold multiples of clinical exposure) will be compatible with the survival of the animals for the span of the carcinogenicity study. It appears that a low-dose of 30 mg/kg/day (approximately 4-fold multiples of clinical exposure) and a mid-dose of 100 mg/kg/day (8-fold multiples of clinical exposure) are also appropriate.

Based on the results of the 2- and 13-week studies, the sponsor's proposed high- (200 mg/kg/day), mid- (100 mg/kg/day) and low-(30 mg/kg/day) doses appear to be appropriate for the 2-year carcinogenicity study in CD-1 mice.

CAC Concurrence (y/n):

CAC Recommendations:

Comments:

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Dose Selection and Protocol for the 2-Year Oral Carcinogenicity Study in CD-1 Mice

Proposed Study: The sponsor has proposed to conduct a 2-year oral carcinogenicity study with SB-497115-GR in CD-1 mice (5-6 weeks of age and 20-45 g body weight). SB-497115-GR (Lot No. TPO-E-01C) will be administered daily by oral gavage at 30, 100, and 200 mg/kg/day at a dose volume of 10 ml/kg. The vehicle control animals will receive aqueous 2% hydroxypropyl methylcellulose with 0.2% sodium lauryl sulfate. There will be 60 mice/sex/group in the carcinogenicity portion of the study. In the toxicokinetic portion of the study, there will be 36 mice/sex/group (Control: 6 mice/sex/group). The study design is shown in the following table (from Vol. 2 of 3, page 11 of sponsor's submission).

GROUP DESIGNATION AND DOSE LEVELS				
Group	No. of Animals		SB-497115-GR	SB-497115-GR
	Male	Female	Dose Level ^{a,b} (mg/kg/day)	Dose Concentration ^{a,b} (mg/ml)
Carcinogenicity Animals				
1 (Control) ^c	60	60	0	0
2 (Low)	60	60	30	3
3 (Mid)	60	60	100	10
4 (High)	60	60	200	20
Toxicokinetic Animals				
5 (Control) ^c	6	6	0	0
6 (Low)	36	36	30	3
7 (Mid)	36	36	100	10
8 (High)	36	36	200	20

a. The dose volume will be 10 ml/kg.
b. Dose concentrations will be adjusted for salt form and purity using a correction factor specific to each batch of test article.
c. Animals in the control groups will receive the control material: (2% hydroxypropylmethylcellulose with 0.2% sodium lauryl sulfate in R/O water) only.

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Three animals/sex from Groups 6 through 8 will be bled at predose, and at 1, 2, 4, 8, and 24 hours postdose). Blood will be collected from all animals in Group 5 (control) at 4 hours post dose.

Animals will be observed daily for mortality and clinical signs of toxicity. Body weights will be recorded at least twice prior to treatment (Week-1), Day 1 prior to dosing, weekly for weeks 1 through 16, and every 4 weeks thereafter. Food consumption will be recorded beginning at Week-1, weekly for weeks 1 through 16, and every 4 weeks thereafter. Hematology assessment will be conducted on all animals at scheduled necropsy. A full necropsy will be performed on all main study animals including dead or moribund animals. The following tissues or organs will be examined histologically from all main study animals: adrenal, aorta, brain, cecum, cervix, clitoral gland, colon, duodenum, epididymides, esophagus, eye, femur, gallbladder, harderian gland, heart, ileum, jejunum, kidneys, larynx, lesions, liver, lungs, lymph nodes (mandibular, mesenteric), mammary gland (inguinal), nasal cavities and nasopharynx with skull, optic nerves, ovary, pancreas, parathyroid, pituitary, preputial gland, prostate, rectum, salivary gland (mandibular, parotid and sublingual), sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, thoracic, and lumbar), spleen, sternum with bone marrow, stomach, testes,

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thymus, thyroid, tongue, trachea, urinary bladder, uterus, and vagina. It is to be mentioned here that the sponsor stated (Vol. 2 of 3, page 1) "when survival approaches 25 animals in any one treatment group, early termination will be considered". It appears that 42% survival (25 of 60 animals) is still adequate to continue the study. The sponsor should be asked to consult the Division if the survival falls below 25%.

Comments: The sponsor's proposed high dose of 200 mg/kg/day appears to be appropriate for the following reasons: 1) mortality observed at 300 mg/kg/day in a 14-day toxicity study and 2) absence of any toxicity at 100 mg/kg/day in the 13-week study. However, sponsor's contention for early termination based on 42% (25 of 60 initial animals) survival is not acceptable. It appears that 42% survival (25 of 60 animals) is still adequate to continue the study. The sponsor should be asked to consult the Division if the survival falls below 25%.

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TOXICOLOGY:

Study Title: 14-Day Oral Toxicity Study in Mice

Key Study Findings: The animals were tested at 0, 30, 100 and 300 mg/kg/day (oral, gavage) for 14 days. Treatment-related mortality was observed at 300 mg/kg/day. The NOAEL appeared to be 30 mg/kg/day in both sexes. The target organ of toxicity appeared to be the liver, kidney, lung and stomach.

Report No./Study No.: CD2003/00476/00

Volume #, and Page #: Vol. C4.1, Page # 8

Conducting Laboratory and Location: Information not provided by the sponsor.

Date of Study Initiation: November 21, 2000

GLP Compliance: A statement of compliance was included.

QA Report: yes () no (X). This study was not subject to any QA monitoring.

Drug, Lot #, and % Purity: SB-497115-GR, F033082, 99.3%

Formulation/Vehicle: Suspension/2% hydroxypropyl methylcellulose with 0.2% sodium lauryl sulfate

Methods:

Dosing:

Species/Strain: CD-1 Mice

No./Sex/Group or Time Point (Main Study): 6/sex/group

Satellite Groups Used for Toxicokinetics: Group 5 (30 mg/kg/day) and 6 (100 mg/kg/day), n = 3/sex/timepoint

Age: 11 weeks

Weight: 34-35 g (approximate)

Doses in Administered Units: 0, 30, 100 and 300 mg/kg/day. The high dose was not tolerated and all animals at 300 mg/kg/day (Group 4) were necropsied on Day 10. The study design is shown below.

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Group	Dose (mg/kg/day)	Concentration (mg/ml)	No. of Animals	
			Main Study	Toxicokinetic
1	0	0	6M/6F	
2	30	3	6M/6F	
3	100	10	6M/6F	
4	300	30	6M/6F	
5	30	3		3M/3F
6	300	30		3M/3F

Route, Form, and Volume: Oral, suspension, and 10 ml/kg

Observations and times:

Clinical Signs: Information not provided

Body Weights: Body weights were recorded on a daily basis

Food Consumption: Not recorded

Hematology: Not performed

Clinical Chemistry: Not performed

Urinalysis: Not performed

Gross pathology: Not performed

Organs weighed: Only the liver was weighed.

Histopathology: The following organs/tissues were examined for histopathology from all animals: skin, thymus, skeletal muscle, lung, heart, liver, esophagus, stomach, kidney, testes, ovary, and adrenal.

Toxicokinetics: Group 5 and 6 animals were bled on Day 14 at 0, 0.5, 1, 2, 4, 8, 12 and 24 hours posttreatment. Plasma samples were analyzed for SB-497115 using a validated LC/MS/MS method.

Results:

Mortality: In males, mortality was observed at all dose levels including control. Mortality was also observed in females at 100 and 300 mg/kg/day. The following table shows unscheduled deaths:

Sex	Males				Females			
	0	30	100	300	0	30	100	300
Deaths	1	1	1	4	0	0	1	3

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Clinical Signs: Clinical signs were observed at 300 mg/kg/day, which included hypoactivity, hypothermia, hunched posture, unkempt appearance, dehydration and scant feces.

Body Weights: The mean initial and final body weight of the control males was 34.5 g and 32.8 g, respectively. The mean initial and final body weight of control females was 27.7 g and 26.4 g, respectively. There were no treatment-related effects on body weight at ≤100 mg/kg/day in either sex.

Organ Weights: There appears to be a dose-related increase in liver weight. Liver weights were 104% and 116% of control (1.82 g) in males and 113% and 122% of control in females.

Histopathology: Generally, treatment-related histopathological changes were observed at 300 mg/kg/day in the stomach (non-glandular ulcer), liver (centrilobular hypertrophy, sinusoidal pigment deposit, hepatocellular necrosis, peribiliary inflammation), kidney (tubular vacuolation, hepatocellular necrosis) and lung (Clara cell hypertrophy and arterial smooth muscle vacuolation). However, histopathological changes (hepatocellular hypertrophy, hepatocellular necrosis and hepatocellular necrosis) were also observed predominantly in males at 100 mg/kg/day. The histopathological findings are shown in the following table.

Tissue Finding	Males (n = 6)				Females (n = 6)			
	0 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	0 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg
Liver								
Hepatocellular hypertrophy, centrilobular	0	0	3	3/4	0	0	0	2/5
Sinusoidal pigment deposit, bile duct	0	0	0	2/4	0	0	0	5/5
Hepatocellular necrosis, coagulative	0	0	3	3/4	0	0	0	4/5
Hepatocellular vacuolation, panlobular	0	0	0	0/4	0	0	0	3/5
Peribiliary inflammation, mixed	0	0	0	1/4	0	0	0	0/6
Stomach								
Ulcer, non-glandular	0	0	0	1/4	0	0	0	1/5
Kidney								
Tubular vacuolation, cortical	0	0	0	2/4	0	0	0	4/5
Necrosis/regeneration, cortical medullary tubule	0	0	1	2/4	0	0	1	4/5
Lung								

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Hypertrophy, Clara cell	0	0	0	2/4	0	0	0	5/5
Smooth muscle vacuolation, arterial	0	0	0	2/4	0	0	0	3/5

¹ Repeat oral doses of 300 mg/kg/day were not tolerated. All remaining animals were sacrificed on Day 10.

Toxicokinetics: Generally, maximum plasma concentration was reached at approximately 2 hours after treatment. The exposure was almost dose proportional in males and more than dose proportional in females. There was no apparent gender difference in the pharmacokinetic parameters for SB-497511. The pharmacokinetic parameters are shown in the following table (from Vol. C4.1, page 31 of sponsor's submission).

Toxicokinetic Results:			
Plasma SB-497511 Concentration Analysis: Mice plasma samples were analyzed for SB-497511 using a validated analytical method based on protein precipitation, followed by HPLC/MS/MS analysis. The lower limit of quantification for SB-497511 was 10.0 ng/mL, using a 50 µL aliquot of mouse plasma with a higher limit of quantification of 2500 ng/mL. (CD2003/00376/00)			
Parameter (n=3/sex/time point)	Dose Day	Dose Level (mg/kg/day)	
		30	100
Male			
AUC ₍₀₋₂₄₎ (µg·h/mL)	Day 14	290	770
C _{max} (µg/mL)	Day 14	30.5	97.5
T _{max} (h)	Day 14	2.02	2.03
Female			
AUC ₍₀₋₂₄₎ (µg·h/mL)	Day 14	306	1274
C _{max} (µg/mL)	Day 14	44.1	134
T _{max} (h)	Day 14	2.06	2.06

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Summary: In a 14-Day oral (gavage) toxicity study in the CD-1 mouse, animals were administered SB-794115 at 0, 30, 100 and 300 mg/kg/day. Treatment-related mortality was observed at 300 mg/kg/day in both sexes. The NOAEL appeared to be 30 mg/kg/day in both sexes. The target organ of toxicity appeared to be the liver (centrilobular hypertrophy, sinusoidal pigment deposit, coagulative hepatocellular necrosis, and peribiliary inflammation), kidney (tubular vacuolation, necrosis/regeneration of cortical medullary tubule), stomach (non-glandular ulcer) and lung (Clara cell hypertrophy and arterial smooth muscle vacuolation).

Study Title: 2-Week Oral Gavage Tolerability and Toxicokinetic Study in CD-1 Mice

Key Study Findings: In a 14-Day oral (gavage) exploratory tolerability and toxicokinetic study in the CD-1 mouse, animals were administered SB-794115-GR at 0, 150 and 200 mg/kg/day. There were no treatment-related clinical signs or body weight changes. The sponsor did not

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conduct any histopathological examinations of any organ or tissue. There was no apparent gender difference in the systemic exposure (C_{max} and AUC_{0-24h}).

Report No./Study No.: CD2004/00836/00

Volume #, and Page #: Vol. C4.1, Page # 73

Conducting Laboratory and Location: _____

Date of Study Initiation: June 14, 2004

GLP Compliance: This study is an exploratory study and was not conducted according to 21CFR58.

QA Report: yes () no (X)

Drug, Lot #, and % Purity: SB-497115-GR, Batch/Lot No. TPO-E-01C. Purity data not provided.

Formulation/Vehicle: Suspension/2% hydroxypropyl methylcellulose with 0.2% sodium lauryl sulfate

Methods:

Dosing:

Species/Strain: CD-1 Mice

No./Sex/Group or Time Point (Main Study): 6/sex/group

Satellite Groups Used for Toxicokinetics: Group 4 (150 mg/kg/day) and 5 (200 mg/kg/day), n = 18/sex/group

Age: 6 weeks

Weight: 20.4-28.2 g

Doses in Administered Units: 0, 150, 200 mg/kg/day. The doses were selected based on the results of the previous study (CD2003/00476/00) in CD-1 mice at 30, 100 and 300 mg/kg/day. Based on the mortality and organ toxicity observed at 300 mg/kg/day, the doses for the current study was selected as 150 and 200 mg/kg/day.

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Group	Dose (mg/kg/day)	Concentration (mg/ml)	No. of Animals	
			Main Study	Toxicokinetic
1	0	0	6M/6F	
2	150	15	6M/6F	
3	200	20	6M/6F	
5	150	15		18M/18F
6	200	20		18M/18F

Route, Form, and Volume: Oral, suspension, and 10 ml/kg

Observations and times:

Clinical Signs: Animals were observed for clinical signs on a daily basis.

Mortality: A mortality check was performed near the start and end of each working day.

Body Weights: Body weights were recorded on a daily basis.

Food Consumption: Food consumption was recorded on a daily basis.

Hematology: None

Clinical Chemistry: None

Urinalysis: None

Gross Pathology: None

Organs Weighed: None

Histopathology: None

Toxicokinetics: Blood samples were collected from toxicokinetic animals during Day 14 at 0, 1, 2, 4, 8 and 24 hours after treatment. Plasma samples were analyzed for SB-497115 using a validated LC/MS/MS method.

Results:

Mortality: There was no mortality.

Clinical Signs: There were no significant treatment-related clinical observations.

Body Weights: The mean initial and final body weight of the control males was 29.0 g and 33.6 g, respectively. The mean initial and final body weight of control female was 22.7 g and 27.7 g, respectively. There were no treatment-related effects on body weight in either sex.

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Toxicokinetics: Generally, maximum plasma concentration was reached at approximately 4 hours after treatment. There was no apparent gender difference in the systemic exposure (C_{max} and AUC_{0-24h}). Mean exposure was slightly higher at 200 mg/kg/day compared to 150 mg/kg/day. Systemic exposure increased 1.3-fold in males and 1.6-fold in females with a 1.3-fold increase in dose. The toxicokinetic parameters for SB-497115 are shown in the following table (from Vol. C4.1, page 124 of sponsor's submission).

Table 1 Toxicokinetic Parameters for SB-497115 Derived from Composite Plasma Concentration-Time Results from Male and Female Mice Following Oral Administration of SB-497115-GR for 2 Weeks

Dose level (mg/kg/day)	Sex	AUC _{0-24h} (ng.h/mL)	C _{max} (ng/mL)	T _{max} (h)
150	Male	818276	89815.2	4.08
	Female	1083516	123807.8	4.08
200	Male	1026824	121517.8	4.11
	Female	1687917	162844.8	4.12

Summary: In a 14-Day oral (gavage) tolerability and toxicokinetic study in the CD-1 mouse, animals were administered SB-794115-GR at 0, 150 and 200 mg/kg/day. There were no treatment-related mortality, clinical signs and body weight changes. There was no apparent gender difference in the systemic exposure (C_{max} and AUC_{0-24h}). Since this was an exploratory study, the sponsor did not conduct any histopathological examinations of any organ and tissue. As a result, histopathological consequences of 150 and 200 mg/kg/day dose could not be determined from this study.

Study Title: 13-Week Oral Toxicity Study in Mice

Key Study Findings: In a 13-Week oral (gavage) toxicity study in the CD-1 mouse, animals were administered SB-794115-GR at 0, 10, 60 and 100 mg/kg/day. The NOAEL appeared to be 100 mg/kg/day in both sexes. The target organ of toxicity could not be identified in the absence of any organ toxicity at any of the tested doses. The dose selection does not appear to be appropriate, as the highest tested dose did not produce any toxicity. It appears that the sponsor could have tested higher doses.

Report No. /Study No.: CD2004/00627/00

Volume #, and Page #: Vol. C4.1 (3.3), Page # 1

Conducting Laboratory and Location: _____

Date of Study Initiation: November 25, 2003

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GLP Compliance: A statement of compliance was included.

QA Report: yes (X) no ().

Drug, Lot #, and % Purity: SB-497115-GR, TPO-E-01C, 99.3%

Formulation/Vehicle: Suspension/2% hydroxypropyl methylcellulose with 0.2% sodium lauryl sulfate

Methods:

Dosing:

Species/Strain: CD-1 Mice

No./Sex/Group or Time Point (Main Study): 12/sex/group

Satellite Groups Used for Toxicokinetics: Group 5, 6, 7 and 8; n = 21/sex/group

Age: 6 weeks

Weight: Males: 14.3 – 34.4 g; Females: 14.5 – 26.6 g

Doses in Administered Units: 0, 10, 60 and 100 mg/kg/day. The doses were selected based on the results of the previous 2-week studies in CD-1 mice as discussed before. The study design is shown in the following table.

Group	Dose (mg/kg/day)	Concentration (mg/ml)	No. of Animals	
			Main Study	Toxicokinetic
1	0	0	12M/12F	
2	10	1	12M/12F	
3	60	6	12M/12F	
4	100	10	12M/12F	
5	0	0		21M/21F
6	10	1		21M/21F
7	60	6		21M/21F
8	100	10		21M/21F

Route, Form, and Volume: Oral, suspension, and 10 ml/kg

Observations and times:

Clinical Signs: Animals were observed twice daily for clinical signs.

Body Weights: Body weights were recorded on a weekly basis.

Food Consumption: Food consumption was recorded weekly.

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Hematology: Hematology was conducted during Week 14.

Clinical Chemistry: Clinical chemistry was performed during Week 14.

Urinalysis: None

Gross Pathology: Gross pathological examinations were conducted on all animals at necropsy

Organs Weighed: The following organs were weighed from all main study animals: adrenals, brain, heart, kidneys, liver including gallbladder, ovaries, thymus, prostate and testes.

Histopathology: The following organs/tissues were examined for histopathology from control and high dose animals (main study): adrenals, aorta, brain, cecum, cervix, colon, duodenum, epididymis, esophagus, eyes, femur, gallbladder, heart, ileum, jejunum, kidneys, larynx, liver, lungs, lymph nodes (mandibular and mesenteric), mammary gland, ovaries, pancreas, parathyroid, pituitary, prostate, rectum, salivary gland, sciatic nerve, skeletal muscles, skin, spinal cord, spleen, stomach, sternum, testes, thymus, thyroid, tongue, trachea, urinary bladder, uterus, vagina etc.

Toxicokinetics: Blood samples were collected during Week 13 from Group 5-8 animals at 0, 1, 2, 4, 8, 12 and 24 hours postdose.

Results:

Mortality: There were no treatment-related mortalities.

Clinical Signs: No treatment-related clinical signs were observed.

Body Weights: The mean initial and final body weight of the control males was 26.9 g and 38.2 g, respectively. The mean initial and final body weight of control females was 20.6 and 29.6 g, respectively. There were no treatment-related effects.

Food Consumption: The mean initial and final food consumption in control males was 5.4 g/animal/day and 5.91 g/animal/day, respectively. The mean initial and final food consumption in control females was 4.57 and 5.27 g/animal/day, respectively. There were no treatment-related effects.

Ophthalmoscopy: There were no treatment-related ophthalmic effects. Phthisis of the globe in one control male and corneal opacity was noted in two males at 10 mg/kg/day. These effects were not considered as treatment-related.

Hematology: There were no treatment-related hematology findings.

Serum Chemistry: There were no treatment-related clinical chemistry findings.

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Organ Weights: No treatment-related effects were observed in the organ weights.

Gross Pathology: There were no treatment-related macroscopic observations.

Histopathology: There were no treatment-related microscopic observations.

Toxicokinetics: Maximum plasma concentration was reached at 4 hours posttreatment. Generally, drug exposure was more than dose proportional. There was no apparent gender difference in the pharmacokinetic parameters for SB-497115. The pharmacokinetic parameters for SB-497115 are shown in the following table [from Vol. C4.1 (3.3) page 340 of sponsor's submission].

Table 1 Toxicokinetic Parameters for SB-497115 Derived from Composite Plasma Concentration-Time Results from Male and Female Mice Following Oral Administration of SB-497115-GR at 10, 60, and 100 mg/kg/day for 13 Weeks

Sex	Dose Level (mg/kg/day)	During Week	AUC _{0-4h} (ng·h/mL)	C _{max} (ng/mL)	T _{max} (h)
Male	10	13	17933.2	2036.9	4.00
	60	13	48456.6	7206.6	4.00
	100	13	63632.3	8476.9	4.00
Female	10	13	23216.0	3622.1	1.00
	60	13	42347.4	4837.6	4.00
	100	13	66847.7	11364.6	4.00

Summary: In a 13-Week oral (gavage) toxicity study in the CD-1 mouse, animals were administered SB-497115-GR at 0, 10, 60 and 100 mg/kg/day. The NOAEL appeared to be 100 mg/kg/day in both sexes. The target organ of toxicity could not be identified in the absence of any organ toxicity at any of the tested doses. The dose selection does not appear to be appropriate, as the highest tested dose did not produce any toxicity. Higher doses could have been tested, as 200 mg/kg/day dose in the 2-week tolerability did not cause any lethality.

SUMMARY AND EVALUATION:

SB-497115-GR is the *bis*-monoethanolamine (MEA) salt form of SB-497115, a non-peptide, orally active thrombopoietin receptor (TpoR) agonist in development for chronic treatment of thrombocytopenia associated with immune thrombocytopenia purpura (ITP) and chronic liver disease.

In this submission, the sponsor has submitted the rationale or the basis for the dose selection and the protocol for the proposed 2-year oral carcinogenicity study in CD-1 mice at 30, 100, 200 mg/kg/day doses and the reports of 2- and 13-week oral toxicity study in CD-1 mice with SB-

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497115-GR. In addition, the sponsor also submitted reports of genotoxicity and protein binding studies with SB-497115-GR.

The sponsor has proposed to conduct a 2-year oral carcinogenicity study in CD-1 mice with SB-497115-GR at 30, 100, and 200 mg/kg/day doses. The sponsor's dose selection was based on the toxicity endpoints determined from results of the 2- and 13-week oral toxicity study in CD-1 mice, 2-week oral tolerability and toxicokinetic study in CD-1 mice and 13-week oral toxicity study in CD-1 mice.

In a 14-Day oral (gavage) toxicity study in the CD-1 mouse, animals were administered SB-794115-GR at 0, 30, 100 and 300 mg/kg/day. Treatment-related mortality was observed at 300 mg/kg/day in both sexes and all mice at this dose level were sacrificed on Day 10 due to deteriorating clinical conditions. The NOAEL appeared to be 30 mg/kg/day in both sexes. The target organ of toxicity appeared to be the liver (centrilobular hypertrophy, sinusoidal pigment deposit, hepatocellular necrosis, peribiliary inflammation), kidney (tubular vacuolation, necrosis/regeneration of cortical medullary tubule), stomach (non-glandular ulcer) and lung (Clara cell hypertrophy and arterial smooth muscle vacuolation).

In a subsequent 2-week exploratory tolerability and toxicokinetic study in CD-1 mice with SB-497115-GR, doses of 150 and 200 mg/kg/day were found to be well tolerated. The sponsor did not conduct any histopathology evaluations of any organ or tissue. Exposure appeared to be approximately dose-proportional with a mean AUC_{0-24h} of 1358 $\mu\text{g}\cdot\text{h}/\text{ml}$ at 200 mg/kg/day.

In a 13-Week oral (gavage) toxicity study in the CD-1 mouse, animals were administered SB-794115-GR at 0, 10, 60 and 100 mg/kg/day. The NOAEL appeared to be 100 mg/kg/day in both sexes. The target organ of toxicity could not be identified in the absence of any organ toxicity at any of the tested doses. The dose selection does not appear to be appropriate, as the highest tested dose did not produce any organ toxicity. Based on the results of the 14-day tolerability and TK study at 150 and 200 mg/kg/day, it appears that higher doses could have been tested.

Mean protein binding of SB-497115 was >99% in human plasma and 94% in mouse plasma. Both SB-497115 and SB-497115-GR (ethanolamine salt) was found to be positive in L5178Y TK⁺ mouse lymphoma assay. The drug was found to be negative in the Ames assay, unscheduled DNA synthesis (UDS) assay using *ex vivo* rat hepatocytes and *in vivo* rat bone marrow micronucleus assay using oral route. SB-497115 appears to have genotoxic potential.

Using human exposure of 80.9 $\mu\text{g}\cdot\text{h}/\text{ml}$ (mean AUC_{0-24h} in normal male subjects given 10 daily doses of 75 mg SB-497115-GR/day) anticipated exposure margins at 30, 100 and 200 mg/kg/day would be expected to be approximately 3.7-, 8- and 17-fold, respectively.

In summary, 200 mg/kg/day appears to be an acceptable high dose for the proposed carcinogenicity study in CD-1 mice. The sponsor's proposed high dose of 200 mg/kg/day appears to be appropriate for the following reasons: 1) mortality observed at 300 mg/kg/day in a 14-day toxicity study and 2) absence of any toxicity at 100 mg/kg/day in the 13-week study. Based on this information, it appears that the sponsor's proposed high dose of 200 mg/kg/day (approximately 17-fold multiples of clinical exposure) will be compatible with the survival of the

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animals for the span of the carcinogenicity study. It appears that a low-dose of 30 mg/kg/day (approximately 4-fold multiples of clinical exposure) and a mid-dose of 100 mg/kg/day (8-fold multiples of clinical exposure) are also appropriate.

Based on the results of the 2- and 13-week studies, the sponsor's proposed high- (200 mg/kg/day), mid- (100 mg/kg/day) and low-(30 mg/kg/day) doses appear to be appropriate for the 2-year carcinogenicity study in CD-1 mice.

RECOMMENDATIONS: The sponsor's high dose (200 mg/kg/day) selection appears to be appropriate for the proposed 2-year oral carcinogenicity study in CD-1 mice.

Tamal K. Chakraborti, Ph.D. Date
Pharmacologist, HFD-180

Comment:

Jasti B. Choudary, B.V. Sc., Ph.D. Date
Supervisory Pharmacologist, HFD-180

cc:

HFD-180
HFD-181/CSO
HFD-180/Dr. Chakraborti
HFD-180/Dr. Choudary

R/D Init. J Choudary: 10/26/04

Appendix #2: Non-neoplastic and neoplastic incidences in carcinogenicity studies:

A. Mouse:



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(3 MB)

B. Rat



ycpromactScanDoc.
PDF (2 MB)

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