4.2.2 A Single Ascending Dose Tolerance Study of Cs-747 in Healthy Male Volunteers with Pharmacokinetic and Pharmacodynamic Assessment (S001)

Study number: ICR 012372
Principal Investigator: Dr S Freestone, Inveresk Clinical Research
Sponsor: Sankyo Co., International Development Department New Drug Development Division 7-12, Ginza, 2-chome Chuo-ku Tokyo 104 - 8113 Japan
Date of first enrolment: 17 November 1997
Study Completed: 22 December 1997
Phase of Development: I

Objective	To determine the safety and tolerability of CS-747 with pharmacokinetic and pharmacodynamic assessment
Study Design	A randomized, double-blind, ascending oral dose study of CS-747. The actual doses studied were 2.5, 10, 30 and 75 mg. Each group consisted of 6 subjects, (5:1, drug:placebo)
Study Population	Healthy males subjects aged 18-50: planned 36 – analyzed 24
Duration of treatment	Two nights plus one outpatient visit (Groups 1 and 2) or two outpatient visits (Groups 3 and 4)
Investigational Drug	CS-747 oral, 2.5 mg tablets Batch No. G97T03, 5 mg tablets Batch No. G97T04 and 25 mg tablets Batch No. G97T05
Reference Product	Placebo: oral tablets, Batch No. G97T01
Assay	The 3 metabolites (R-95913, R-106583 and R-100932) were in plasma by the LC/APCI-MS/MS (Sankyo Co.)
PK Assessment Plasma Urine	R-95913, R-106583 and R-100932, were measured. Samples: Predose (0), 0.5, 1, 1.5, 2, 4, 6, 8, 12 and 24 h post dose. Samples: predose and 0-12 h post dose from subjects receiving CS-747 at the highest dose level.
Pharmacodynamics: Platelet Aggregation Bleeding Time	Samples at predose, 1, 2, 4, 8, 24 h and Day 7 post dose and at 48 h post dose in the 2 highest dose groups. The maximum % aggregation response to ADP (20 μM), ADP (5 μM) and collagen (2 μg.ml-1) was recorded over a 4 min monitoring period. IVY Nelson method, at screening, predose (0), 4 h and 24 h post dose and at 7 day follow-up.
Statistics	Descriptive statistics have been used to summarise the following data by dose group: demographic details, urinalysis, platelet aggregation, vital signs, bleeding times and adverse events.
Safety	Physical examination, vital signs, electrocardiograph, clinical pathology, urinalysis, fundoscopy, petechiae, coagulation (Prothrombin time, activated prothrombin time, fibrinogen) platelet aggregation adverse events, faecal occult bloods.

Results
Assay

A quantitation method for the concentration of R-95913, R-106583 and R-100932 in human plasma by LC/APCI-MS/MS were investigated using a solid phase extraction as the pretreatment procedure.
First, the ion-monitoring detection in SRM was studied. The [M+H]+ ions (m/z at 332.4 for R-95913, m/z at 336.4 for D4-R-95913 (I. Std. of R-95913), m/z at 364.4 for R-106583 and R-100932 and m/z at 371.4 for D7-R-100932 (I. Std. of R-100932) were selected for monitoring at Q1. After fragmentation, the ion with m/z at 148.9 for both R-95913 and its I. Std. the ion with m/z at 205.9 for R-106583 and R- 100932 and the ion with m/z at 207.9 for the I. Std. (D7-R-100932) showed the highest intensity at Q3. Thus, the quantitation by SRM (Selected reaction monitoring) was conducted by selecting the ions of m/z at 332.4 to 148.9 for R-95913, m/z at 336.4 to 148.9 for its I. Std., m/z at 364.4 to 205.9 for R-106583 and R-100932 and m/z at 371.4 to 207.9 for the I.Std. (D7-R-100932), respectively.

Table 27: Assay Characteristics of Inactive Metabolites in Plasma

Parameter	R100932		R106583		R95913	
Linearity	1.56 ng/mL to 800 ng/mL					
	Inter-batch	Intra-batch	Inter-batch	Intra-batch	Inter-batch	Intra-batch
Precision (CV %)	0.7 to 18.4	0.6 to 10.4	1.3 to 11.9	1.2 to 11.7	0.69 to 15.3	0.4 to 12.1
Accuracy, %	-1.3 to -10.6	-1.9 to 17.8	-1.4 to 15.8	-1.8 to 11.6	-2.1 to 17.1	-2.8 to 16.5
LLOQ	1.56 ng/mL					
Reviewer Comment	The assay characteristics and specificity are poorer than in the other studies, the upper limits of variability in several cases were above 15%. The representative mass-chromatograms are shown.					

Demographics:
Twenty four healthy male subjects with ages ranging from 19 to 48 years completed the study. The mean age was 30.38 years (SD 7.38, range 19.0-48.0 years), mean height was 174.29 cm (SD 8.07, range 152.0-191.0 cm), mean weight was 73.11 kg (SD 9.27, range 59.9-93.2 kg and mean caliper size was 7.38 cm (SD 0.28, range 6.8-8.1 cm).

Pharmacokinetics

Table 28. Pharmacokinetic Parameters of R-95913

Parameter		Dose (mg)			
		2.5	10	30	75
$AUC_{(0\text{-}24\ h)}$ ($ng.h.ml^{-1}$)	Mean	9.23	53.22	157.91	350.79
	SD	5.31	23.77	50.25	80.62
C_{max} ($ng.ml^{-1}$)	Mean	7.37	30.02	90.83	117.02
	SD	2.60	15.43	28.99	61.57
t_{max} (h)	Mean	0.70	0.80	0.60	1.00
	SD	0.27	0.27	0.22	0.35
$MRT_{(0\text{-}24\ h)}$ (h)	Mean	1.12	2.50	2.97	5.15
	SD	0.41	0.98	1.28	2.34

Table 29. Pharmacokinetic Parameters of R106583

Parameter		Dose (mg)			
		2.5	10	30	75
$AUC_{(0\text{-}24\ h)}$ (ng.h.ml^{-1})	Mean	58.39	324.54	969.01	2686.12
	SD	19.54	57.34	293.71	823.75
C_{max} (ng.ml^{-1})	Mean	14.60	71.47	189.40	391.53
	SD	3.63	17.32	52.61	204.82
t_{max} (h)	Mean	0.80	1.10	0.90	1.50
	SD	0.27	0.22	0.22	0.50
$MRT_{(0\text{-}24\ h)}$ (h)	Mean	3.76	6.27	6.44	7.49
	SD	0.54	0.70	0.57	1.31

Table 30. Pharmacokinetic Parameters of R100932

Parameter		Dose (mg)			
		2.5	10	30	75
$AUC_{(0\text{-}24\ h)}$ (ng.h.ml^{-1})	Mean	7.38	62.44	204.07	585.05
	SD	3.59	21.65	39.53	211.30
C_{max} (ng.ml^{-1})	Mean	5.22	28.94	77.80	163.02
	SD	2.41	8.84	17.10	101.19
t_{max} (h)	Mean	1.00	0.90	0.70	1.10
	SD	0.50	0.22	0.27	0.42
$MRT_{(0\text{-}24\ h)}$ (h)	Mean	1.25	2.27	3.20	5.19
	SD	0.60	0.69	0.90	1.94

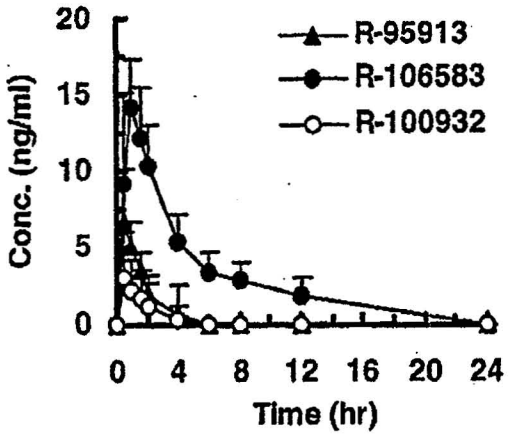

Figure 37 Sponsor's plot of the mean plasma concentrations of 3 metabolites of prasugrel after the 2.5 mg dose of prasugrel.

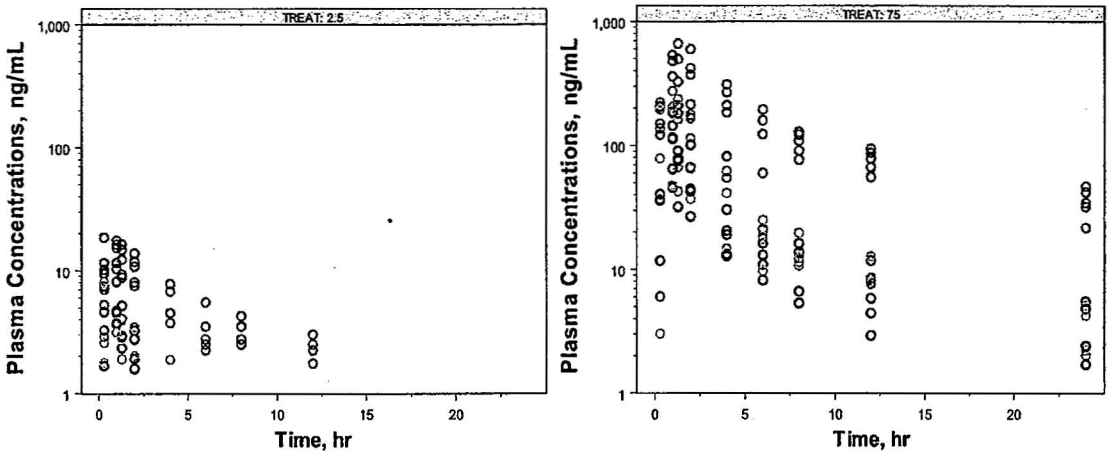

Figure 38 Three metabolites of prasugrel after the 2.5 mg (left) and 75 mg (right) doses of prasugrel.

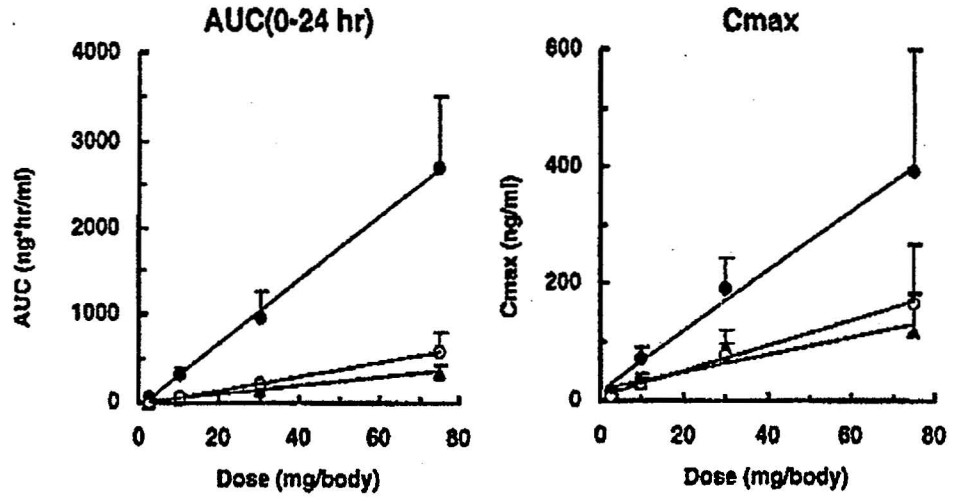

Figure 39. The sponsor's plots for the assessment of linearity of Cmax and AUC0-24.

The sponsor did not report the R values of the linear regressions; therefore, the linearity of pharmacokinetics of the metabolites cannot be evaluated. After the reviewer' request, the sponsor has sent the results of the regression analysis for all analytes Cmax and AUC vs. dose.

Table 31. Results of the Regression analysis for All Analytes

Analyte	R-100932		R-95913		R-106583	
Parameter	Cmax	AUC	Cmax	AUC	Cmax	AUC
R^2	0.597	0.830	0.597	0.897	0.692	0.871

The results indicate that the linearity was poor for the Cmax values and reasonable for the AUC values.

Pharmacodynamics:

The onset of inhibition of platelet aggregation was fast. The mean IPA values at 1 hour after the prasugrel doses of 2.5, 10, 30, and 75 mg were 14, 16, 55, 59% respectively, measured with 5mcM ADP. It peaked at about 2-4 hours and sustained until 24 hours post-dose (25, 29, 72, and 76% respectively). After the dose of 30 mg, the average IPA of 70% was observed at 2 hours post-dose. The further increase of the dose did not significantly change the IPA values.

The reviewer plot of IPA vs time is shown in the figure below.

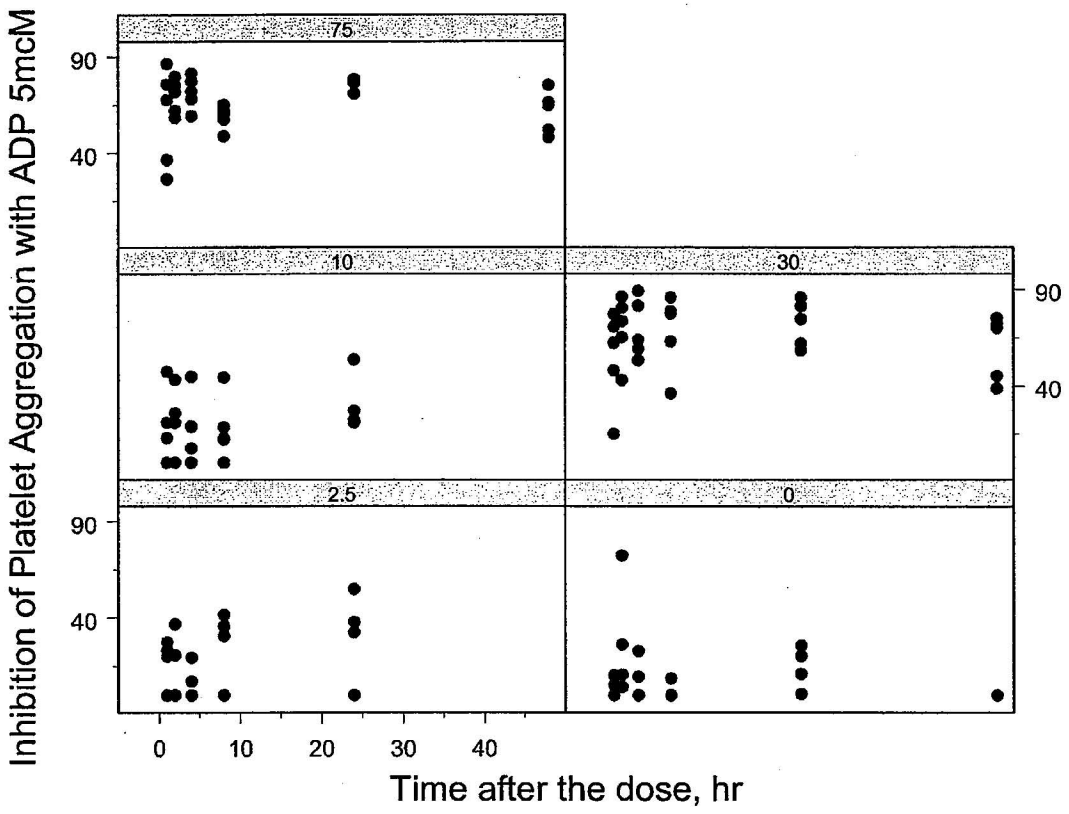

Figure 40. Inhibition of platelet aggregation with ADP 5mcM in individual subjects after the doses of prasugrel of 2.5, 10, 30, 75 mg and placebo

Similar results were observed for the inhibition of platelets aggregation with 20mcM ADP.

Bleeding time also increased with dose. In 2 subjects who received a dose of 75 mg prasugrel, the bleeding did not stop over 30 min observation period.

Sponsor's conclusions:

The pharmacodynamic analysis indicates an inhibition of platelet aggregation induced by either 5 μM or 20 μM ADP in the 30 mg and 75 mg dose groups. There appears to be a rapid onset of effect, inhibition occurring within 1 h of dosing and continuing through 48 h post dose. This suggests an irreversible inhibition by CS-747 and/or its metabolites. Platelet aggregation at Day 7 had returned to normal levels in both these dose groups.

There was an associated prolongation of bleeding times.

Pharmacokinetic analysis shows the observed Cmax and AUC(0-24 h) of the 3 main metabolites of CS-747 increase proportionally to the dose administered from 2.5 mg to 75 mg. Therefore CS-747 was concluded to obey linear pharmacokinetics for the doses tested.

Reviewer Comments

1. The pharmacokinetics of the prasugrel metabolites were close to linear with respect to AUC (R values of 0.83-0.89) but Cmax increased more than proportionally with dose (R values of 0.60-0.69) and therefore, the kinetics were not linear with respect to Cmax at higher doses.
2. The onset of inhibition of platelet aggregation was fast and pronounced already at 1 hour post prasugrel dose. The response peaked at about 2-4 hours and sustained until 24 hours post-dose (25, 29, 72, and 76% respectively). The increase of the dose above 30 mg did not significantly change the IPA values.
3. Bleeding time increased with dose. After a 30 mg dose of prasugrel, one subject stopped bleeding after 21 min. In 2 subjects who received a dose of 75 mg prasugrel, the bleeding did not stop over the 30 min observation period.
4. The use of a 75 mg dose prasugrel does not seem reasonable.

4.2.3 A Double Blind, Placebo Controlled, Multiple Dose Study OF CS-747 Compared with Clopidogrel in Healthy Male Volunteers (S004)

Study number: ICR 014081
Principal Investigator: Dr S Freestone, Inveresk Clinical Research
Sponsor: Sankyo Co., International Development Department New Drug Development Division 7-12, Ginza, 2-chome Chuo-ku Tokyo 104 - 8113 Japan
Date of first enrolment: 27 September 1999
Study Completed: 2 December 1997
Phase of Development: I

Objective	To assess the safety, tolerability and effect on platelet function of 3 doses of CS-747 compared to placebo and clopidogrel when given in multiple doses.
Study Design	A randomized, double blind, placebo controlled, multiple dose study of CS-747 compared with clopidogrel. Each group of the 3 groups of ten subjects comprised of subjects receiving placebo (2), clopidogrel 75 mg (2), CS-747 5 mg(2), CS-747 10 mg (2) and CS-747 20 mg (2) daily.
Study Population	Healthy males subjects aged 18-50: entered 31 – completed 30
Investigational Drug	CS-747 (Batch No. A99T02) 5, 10 and 20 mg tablets
Reference Product	Clopidogrel (Batch No. SD 001 and SE 001) 75 mg Matching CS-747 placebo tablets (Batch No. A99T01)
Dosage and Administration	Dosing was once daily and a total of 10 doses were given to each subject.
Sampling: Blood	0, 1, 2, 4, 6, 8, 12 and 24 hrs post dose on Days 1, 5, and 10. Pre-dosing on Days 3 and 8.
Assay	The 3 metabolites (R-95913, R-106583 and R-100932) were in plasma by the LC/APCI-MS/MS (Sankyo Co.)
PK Assessment Plasma	Prasugrel metabolites: R-95913, R-106583 and R-100932, clopidogrel metabolite (SR26334)
Pharmacodynamics: Platelet Aggregation Bleeding Time	Samples: Day -1, Predose (0), Days 1 and 10: 4, 8, and 24 h post-dose.Days 3, 5, 8 and 12: 4 hours post-dose Additional samples were taken on Days 13, 14, 17 and 24 if aggregation was inhibited by >50% at the previous timepoint. IVY Nelson method, at screening, predose (0), 4 h and 24 h post dose (Days 1, 5, and 10 and pre-dose on Day 8 and 48 hours later. On Days 13, 14, 17 and 24 if prolonged on the previous occasion (mean bleeding time >7 min).

Results
Assay

Table 32: Assay Characteristics of Inactive Metabolites in Plasma

Parameter	R100932	R106583	R95913
Linearity	1.56 ng/mL to 400 ng/mL		

	Inter-batch	Intra-batch	Inter-batch	Intra-batch	Inter-batch	Intra-batch
Precision (CV %)	3.0 to 7.3	1.1 to 3.2	5.3 to 12.6	4.4 to 5.0	2.4 to 8.1	1.9 to 5.4
Accuracy, %	-8.3 to 3.0	-5.0 to -3.0	-6.0 to 0.9	-3.2 to 9.3	-1.9 to 1.8	– 10.5-2.2-
LLOQ	1.56 ng/mL					
Reviewer Comment	The assay characteristics and specificity are acceptable. The representative mass-chromatograms are shown.					

Demographics

The mean (SD) age of the subjects was 31.5 (9.8) years. The mean (SD) height of the subjects was 177.6 (5.8) cm. The mean (SD) weight of the subjects was 79.28 (7.95) kg. All subjects were caucasian males.

Pharmacokinetics

The pharmacokinetic data were not available for the 75 mg dose of CS747. The sponsor presented the PK data for the individual subjects for days 1, 5, and 10 of dosing. Only parameters for Day 1 were summarized by the sponsor (see below).

Table 33. AUC(0-t) and Cmax Values for the 3 Metabolites of CS-747 on Day 1

	Dose of CS-747	AUC (0-t) (ng.h/ml)	Cmax (ng/ml)
R-95913			
	5 mg	18.08	10.73
	10 mg	44.42	18.24
	20 mg	102.95	39.69
R-100932			
	5 mg	51.70	9.02
	10 mg	72.58	15.33
	20 mg	151.83	32.34
R-106583			
	5 mg	253.50	34.55
	10 mg	415.83	60.96
	20 mg	782.00	120.70

The reviewer plotted the plasma concentration data. An example for R-1006583 in plasma after a dose of 10 mg of CS-747 is shown below. There is no obvious dose accumulation on Day 10.

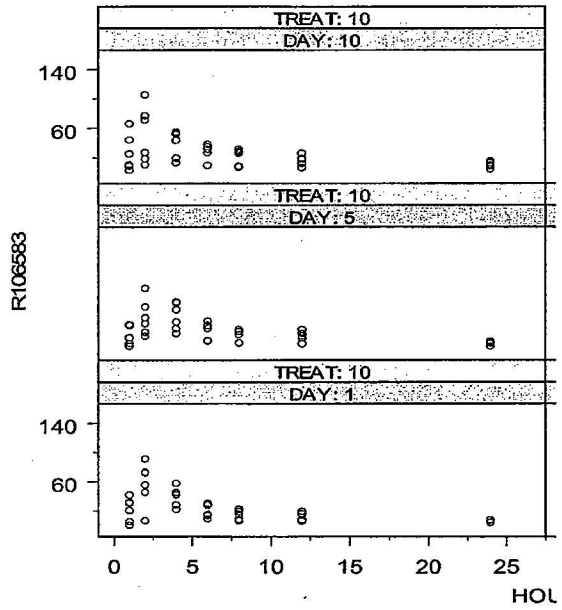

Figure 41. R-1006583 plasma concentrations vs time after the dose of 10 mg of CS-747

The sponsor reported that there was an increase in the mean AUC (0-t) for R-95913 values (about 1.5-fold) between Day 1 and Day 10 but the increase for R- 100932 and R-106583 was less marked. Both AUC(0-t) and Cmax of all 3 metabolites of CS-747 increased with dose as expected. For R-100932 and R- 106583 the increase was slightly less than dose-proportional, but for R-95913 the increase in AUC(0-t) was slightly greater than dose proportional (the regression analysis results were not shown).

Pharmacodynamics

Bleeding Times

The median maximum bleeding time values for placebo, 5 mg CS-747, 10 mg CS-747, 20 mg CS-747 and 75 mg clopidogrel on Day 1 were 195.0 s, 186.5 s, 175.0 s, 282.5 s, and 235.0 s, respectively and on Day 10 were 225.5 s, 292.5 s, 636.5 s, 1032.5 s, and 400.0 s, respectively. The values for all active dose groups on Day 10 were higher than for placebo and there was an upward trend with dose of CS-747.

For Day 1, none of the pairwise differences between the treatments was statistically significant at the 5% level (Kruskal-Wallis analysis, p=0.35). For Day 10, the overall p-value was <0.001: 10 mg CS-747, 20 mg CS-747 and 75 mg clopidogrel were all significantly higher than placebo at the 5% level (p<0.001, p<0.001 and p=0.021 respectively), and 10 mg CS-747 and 20 mg CS-747 were both significantly higher than 5 mg CS-747 at the 5% level (p=0.015 and p=0.006 respectively). None of the other pairwise comparisons was statistically significant at the 5% level.

The Jonckheere-Terpstra non-parametric dose related (two-sided) trend test for Day 1 was not statistically significant at the 5% level (p=0.54) however the test for Day 10 was statistically

significant at the 5% level (p=0.001) showing an increase in maximum bleeding times with dose of CS-747.

Platelet Aggregation

Summary statistics for platelet aggregation mean responses (ADP 5 μM, ADP 20 μM and collagen 1 μg.ml-1, collagen 2 μg.ml-1, adrenaline 5 μM, arachidonate 0.75 mM and adrenaline 10 μM were calculated.

The arithmetic mean (SD) minimum platelet aggregation response with ADP (5 μM) values for placebo, 5 mg CS-747, 10 mg CS-747, 20 mg CS-747 and 75 mg clopidogrel on Day 1 were 52.8% (6.6%), 48.0% (13.7%), 41.7% (10.0%), 22.8% (4.4%) and 38.7% (7.8%), respectively and on Day 10 were 47.0% (15.5%), 19.8% (6.6%), 14.7% (8.4%), 9.5% (1.5%) and 25.2% (6.3%), respectively. The values for all dose groups were lower than for placebo and there was a downward trend with dose of CS-747 on both days. The test for a dose related trend using a linear contrast for Day 1 was statistically significant at the 5% level ($p<0.001$) showing a decrease in minimum platelet aggregation response with dose of CS-747. For Day 10, the trend test was also statistically significant at the 5% level ($p<0.001$), again showing a decrease in minimum platelet aggregation response with dose of CS-747.

For the same test with ADP 20 μM similar trend were observed. In general, the significant trend test showed a decrease in minimum platelet aggregation with dose of CS-747 in the following order: CS-747 20 mg > CS-747 10 mg > CS-747 5 mg > clopidogrel 75 mg > placebo.

Reviewer Comments:

1. The presentation of the results of this study was poor. The sponsor did not include any graphic exploration of data. The sponsor did not attempt to correlate plasma concentrations data with the PD response.
2. The sponsor did not assess the linearity of pharmacokinetics after multiple doses of prasugrel up to 75 mg.
3. A statistically significant increase in maximum bleeding times with dose of CS-747 was observed.
4. With ADP (20 μM) agonist there was a statistically significant decrease in minimum platelet aggregation at Day 10 demonstrating a clear order of potency of the investigational products as follows: CS-747 20 mg > CS-747 10 mg > CS-747 5 mg > clopidogrel 75 mg > placebo.