3 DETAILED LABELING RECOMMENDATIONS

GENERAL

The Agency considered that the overall information regarding Clinical Pharmacology provided in the original NDA 22-307 was appropriate.

CLINICAL PHARMACOLOGY LABELING COMMENTS

Highlights Section:

b(4)

b(5)

4 Page(s) Withheld

_____ Trade Secret / Confidential (b4)

X Draft Labeling (b4)

X Draft Labeling (b5)

_____ Deliberative Process (b5)

4 APPENDIX II:

4.1 Individual In Vitro Study Reviews

4.1.1 In Vitro Protein Binding of Metabolites OF CS-747 (Report No. ATR-151-053)

Investigator: Atsushi Kurihara, Ph.D., Drug Metabolism and Pharmacokinetics Research Laboratories, Sankyo Co., Ltd., 2-58 Hiromachi 1-chome, Shinagawa-ku, Tokyo 140-8710, Japan
Date of completion: June 27 2005

Objectives	To determine the extent of the protein binding ratios of inactive metabolites of CS-747, i.e., R-95913, R-106583, R-100932, R-119251 in plasma and R-138727 in 4% Human serum albumin (HAS)
Methods	Unbound fractions of the main metabolites of CS-747 in plasma, R-95913, R-119251, R-100932 and R-106583, were measured by an ultracentrigufation method. Rat, dog and human plasma at the concentrations of 50, 100 and 500 ng/mL or 100, 500 and 1,000 ng/mL Each mixture (final volume: 1 mL) was incubated at 37°C for 5 min. Then, centrifuged and ultracentrifuged at 436,000 g for 140 min at 15°C The protein-containing fraction (lower layer), protein-free fraction (middle layer) and lipoprotein-containing fraction (upper layer) separated. [redacted] (for R-95913, R-100932, R-106583 and R-138727 assay) or [redacted] (for R-119251 assay) as the internal standard. The mixtures were vortexed and centrifugated at 14,000 rpm for 3 min at 4 °C. The protein binding ratio in rat, dog and human plasma was calculated. HSA solution at 4% was prepared with sodium phosphate buffer (pH 7.4). R-138727 was mixed with 4% HSA at concentrations of 50, 100 and 500 ng/mL, and then each mixture (final volume: 1 mL) was incubated at 37°C for 5 min. After the incubation, the protein-free fraction and total concentration of R-138727 were measured as described above for the other four inactive metabolites.
Assay	LC-MS for the total plasma concentrations of R-95913, R-106583, R-100932, R-119251. Unbound fraction of R-138727 (active metabolite of CS-747) in 4% HSA was also measured by the ultracentrifugation method.

b(4)

Results
The protein binding ratios of R-119251, R-100932, R-106583 and R-95913 are shown in Tables 3, 4 and 5. The result of binding with HSA of R-138727 are also summarized in Table 6. In plasma of each species, the protein binding ratios of R-95913, R-106583 and R-100932 were more than 80% at concentrations of 50, 100 and 500 ng/mL. Binding ratio of R-119251 was 71-77% in rat, 26-36% in dog and 76-77% in human plasma at 100, 500 and 1000 ng/mL. To

evaluate the plasma protein binding of the active metabolite, R-138727, commercially available purified HSA was used since this metabolite has been reported to be unstable in the plasma of each species.3) As a result, R-138727 was proved to be bound to 4% HSA with a binding ratio of 98% at concentrations of 100 and 500 ng/mL.

Table 9. Protein binding ratios of R-95913, R-100932, R-106583 and R-119251 in rat (left panel) and dog (right panel) plasma

<R-95913>

	Protein binding ratio (%)		
	50 ng/mL	100 ng/mL	500 ng/mL
No. 1	86.95	86.65	85.98
No. 2	89.20	87.52	84.84
No. 3	86.69	86.90	84.54
Mean	**87.61**	**87.02**	**85.12**
S.D.	1.38	0.45	0.76

<R-95913>

	Protein binding ratio (%)		
	50 ng/mL	100 ng/mL	500 ng/mL
No. 1	89.14	88.58	88.25
No. 2	93.07	93.27	92.53
No. 3	85.58	87.42	84.57
Mean	**89.26**	**89.76**	**88.45**
S.D.	3.75	3.10	3.98

<R-100932>

	Protein binding ratio (%)		
	50 ng/mL	100 ng/mL	500 ng/mL
No. 1	93.57	92.14	92.13
No. 2	92.87	92.69	91.92
No. 3	92.15	92.61	92.10
Mean	**92.86**	**92.48**	**92.05**
S.D.	0.71	0.30	0.11

<R-100932>

	Protein binding ratio (%)		
	50 ng/mL	100 ng/mL	500 ng/mL
No. 1	84.94	85.17	79.35
No. 2	88.56	87.13	83.61
No. 3	81.89	77.86	76.69
Mean	**85.13**	**83.39**	**79.88**
S.D.	3.34	4.89	3.49

<R-106583>

	Protein binding ratio (%)		
	50 ng/mL	100 ng/mL	500 ng/mL
No. 1	85.13	82.48	83.63
No. 2	82.84	82.81	82.30
No. 3	81.54	82.77	82.94
Mean	**83.17**	**82.69**	**82.96**
S.D.	1.82	0.18	0.67

<R-106583>

	Protein binding ratio (%)		
	50 ng/mL	100 ng/mL	500 ng/mL
No. 1	83.93	84.87	80.07
No. 2	86.51	84.74	82.24
No. 3	83.61	80.66	79.85
Mean	**84.68**	**83.42**	**80.72**
S.D.	1.59	2.39	1.32

<R-119251>

	Protein binding ratio (%)		
	100 ng/mL	500 ng/mL	1000 ng/mL
No. 1	68.78	76.82	76.39
No. 2	71.21	76.38	77.51
No. 3	73.06	75.80	77.60
Mean	**71.02**	**76.33**	**77.17**
S.D.	2.15	0.51	0.67

<R-119251>

	Protein binding ratio (%)		
	100 ng/mL	500 ng/mL	1000 ng/mL
No. 1	32.46	28.12	26.53
No. 2	33.21	21.50	21.86
No. 3	40.95	31.37	30.70
Mean	**35.54**	**27.00**	**26.36**
S.D.	4.70	5.03	4.42

Table 10 Protein binding ratios of R-95913, R-100932, R-106583 and R-119251 in human plasma

<R-95913>

	Protein binding ratio (%)		
	50 ng/mL	100 ng/mL	500 ng/mL
No. 1	93.60	93.72	94.19
No. 2	94.12	94.15	96.26
No. 3	95.42	93.70	96.31
Mean	**94.38**	**93.86**	**95.59**
S.D.	0.94	0.25	1.21

<R-100932>

	Protein binding ratio (%)		
	50 ng/mL	100 ng/mL	500 ng/mL
No. 1	94.98	91.21	91.40
No. 2	93.45	89.51	89.70
No. 3	93.52	92.32	90.89
Mean	**93.98**	**91.01**	**90.66**
S.D.	0.86	1.42	0.87

<R-106583>

	Protein binding ratio (%)		
	50 ng/mL	100 ng/mL	500 ng/mL
No. 1	N.A.	96.79	95.28
No. 2	N.A.	94.15	94.26
No. 3	N.A.	95.88	94.27
Mean	N.A.	**95.61**	**94.60**
S.D.	N.A.	1.34	0.59

<R-119251>

	Protein binding ratio (%)		
	100 ng/mL	500 ng/mL	1000 ng/mL
No. 1	74.67	75.29	78.23
No. 2	76.54	75.74	74.89
No. 3	75.32	76.41	76.03
No. 4	78.50	77.07	78.29
Mean	**76.26**	**76.13**	**76.86**
S.D.	1.68	0.78	1.68

Table 11 Protein binding ratios of R-138727 in 4% HSA

	Protein binding ratio (%)		
	50 ng/mL	100 ng/mL	500 ng/mL
No. 1	N.A.	97.96	98.06
No. 2	N.A.	97.96	97.91
No. 3	N.A.	97.95	97.99
Mean	N.A.	**97.96**	**97.99**
S.D.	N.A.	0.01	0.08

4.1.2 Exploratory Studies on the in Vitro Biotransformation of Prasugrel (LY640315) by Human Carboxylesterases 1 and 2 (Report # 2007IV-EI002)

Reported by Eric T. Williams
Conducted by Nagy A. Farid, Everett J. Perkins, G. Douglas Ponsler, and Eric T. Williams
Eli Lilly and Company Lilly Corporate Center Indianapolis, IN 46285
Date: October 2007

Objectives	To examine in vitro the potential involvement of human carboxylesterases, hCE1 and hCE2, the dominant forms in the liver and intestinal tract, respectively, in the bioactivation of prasugrel.
Investigated Compound	Prasugrel, R-95913, and R-104731 (Sankyo Co., Ltd) The enzymes hCE1 and hCE2, were expressed and purified by the Integrative Biology Protein Expression group at Eli Lilly.
Incubation at 37°C	Prasugrel (in 2% acetonitrile) solutions at concentrations from 0.855 to 109 μM pre-incubated (1 min). The enzyme, hCE1 (1 μg/mL) or hCE2 (0.25 μg/mL) pre-incubated with buffer (5 min) and added to the prasugrel. After 1, 2, 3, and 6 min reaction terminated by addition to 100 μL of acetonitrile containing 2 μg/mL of R-104731, a d4-labeled R-95913, as the internal standard.
Assay	LC-MS/MS
Data Analyses	Hydrolysis reaction rates (nmol of product/min/μg of protein) were calculated from the linear portion of the concentration versus time curve. Fittings: WinNonlin (Pharsight Corp.; Mountain View, CA). Michaelis-Menten, substrate inhibition, and Hill kinetic models with differing weighting were used.

Prasugrel is a prodrug that requires a two-stage biotransformation for the formation of the active metabolite. The first step is the hydrolysis of the ester bond to yield R-95913 (Farid et al. 2007a) which may be the result of the carboxylesterases (CEs). This is followed by oxidation of R-95913 which has been shown to be mediated primarily by CYP3A and CYP2B6 (Rehmel et al. 2006).

The carboxylesterases are a multigene family that hydrolyze compounds containing an ester, amide, or thioester linkage. Two primary forms exist in humans, hCE1 and hCE2. Carboxylesterases are broadly expressed throughout the body. Both forms have greatest mRNA expression in the liver, while extrahepatic expression differs (Satoh et al. 2002). For hCE1, the liver-dominant form, extrahepatic mRNA expression observed in decreasing order are the stomach, testis, kidney, spleen, and colon. The intestinal-dominant form, hCE2, has extrahepatic mRNA expression in decreasing order in the colon, small intestine, and heart.

Although hCE1 and hCE2 have overlapping substrate recognition, selectivity for substrates has been observed (Satoh et al. 2002). Two products result from ester hydrolysis, an alcohol and an acyl moiety. In general, hCE1 prefers substrates with a large acyl moiety, while hCE2 prefers substrates with a large alcohol substituent. Based upon the known substrate recognition of these two carboxylesterases, prasugrel would be predicted to be a preferred substrate for hCE2.

This study aims to show the potential involvement of hCE1 and hCE2, the dominant forms in the liver and intestinal tract, respectively, in the bioactivation of prasugrel.

Results

The human carboxylesterases hCE1 and hCE2 hydrolyzed prasugrel to R-95913. Figure 24(A) depicts the fit of standard Michaelis-Menten kinetics to the data for hCE1, with an apparent Km of 9.25 ± 0.78 μM and an apparent Vmax of 0.725 ± 0.035 nmol of R-95913/min/μg of protein. The results obtained using hCE2 were not fit to any standard kinetic model, as seen in (B). The sponsor described the data using 2 models. The first data set (prasugrel concentrations of 0.855 and 40.5 μM) was described with the Hill equation, Ks of 11.1 ± 2.8 μM, and Vmax of 19.0 ± 2.8 nmol of R-95913/min/μg of protein, and N= 1.42 ± 0.12. The second portion of the curve resembles an inhibition plot and as such the data between 27.4 and 109 μM was modeled for inhibition to yield an apparent IC50 of 76.5 ± 2.7 μM.

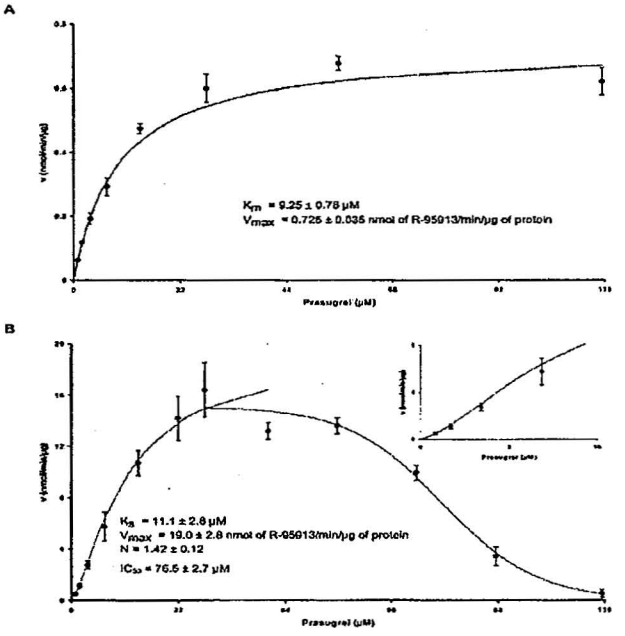

Figure 24. Formation of R-95913 by hCE1 (A) and hCE2 (B). The points with the error bars represent the average and standard error, while the lines represent the best model fit of their respective kinetic models. Inset shows the sigmoidicity of the Hill kinetics.

The hydrolysis of prasugrel with both hCE1 and hCE2 have similar Km and Ks values. The hydrolysis activity (Vmax) of hCE2 was about 26-times higher than that of hCE1. This is consistent with the published data regarding carboxylesterase substrate preferences (Satoh et al. 2002).

Comment:

1. The raw data of this study were not available for the review.
2. This study determined the ability of expressed and purified human carboxylesterases 1 (hCE1) and 2 (hCE2), the dominant forms in the liver and intestine, respectively, to hydrolyze prasugrel to R-95913 in vitro. The study showed that these human carboxylesterases efficiently catalyze the conversion of prasugrel to R-95913, with 26 times the rate of turnover by hCE2 compared to hCE1.

4.1.3 Examination of Effects of LY640315 on CYP1A2 and CYP3A in Primary Cultures of Human Hepatocytes

Investigator: J.L. Fayer, Eli Lilly, Indianapolis, IN 46285, USA
Date: August 2005

Objectives	To examine the ability of R-95913 to induce the catalytic activities associated with CYP1A2 and CYP3A in primary cultures of human hepatocytes from four separate donors.
Method of incubation	Primary cultures of human hepatocytes were treated for 72 hours with R-95913 at concentrations ranging from 0.1 to 100 μM, and the effects of treatment on catalytic activities associated with CYP1A2 and CYP3A were compared to those activities in vehicle control cultures (0.1% DMSO). Omeprazole and rifampicin were used to demonstrate induction responses for CYP1A2 and CYP3A activity. The cells were incubated with 100 mcM testosterone or 2mcM 7-ethoxy-resorufin (with 3mM salicylamide) in HMM for 30 minutes. Cells were harvested by scraping in 1.0 mL 100 mM potassium phosphate buffer (pH 7.4), and were stored at approximately -80°C prior to determination of protein content.
Assay	HPLC with UV detection: the Lilly Global Chromatography Data System (GCDS), version 2, Eli Lilly and Company.

Results

At 0.1, 1.0, 10, and 100 μM concentrations of R-95913, preparations in 3 human hepatocytes (HH) showed some changes in CYP1A2- mediated EROD activity, examples in 2 HH are shown below:

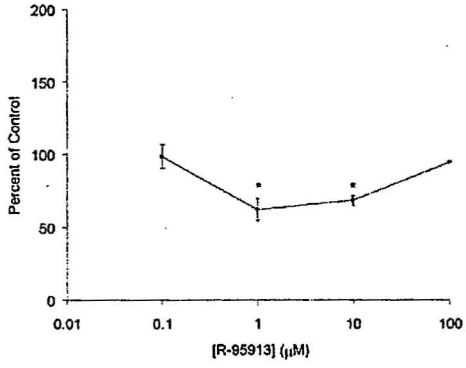

Figure 1: The effect of R-95913 on ethoxyresorufin O-deethylase activity in human hepatocyte preparation HH994.

Activities are expressed as percent of control ± standard error of the mean. Mean control activity (n = 3) was 0.212 pmol/min/mg, and omeprazole-treated cells had 226-fold greater activity than controls.

*P-value less than 0.05.

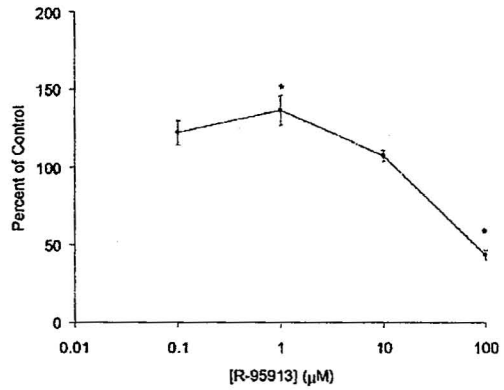

Figure 2: The effect of R-95913 on ethoxyresorufin O-deethylase activity in human hepatocyte preparation HH997.

Activities are expressed as percent of control ± standard error of the mean. Mean control activity (n = 3) was 0.787 pmol/min/mg, and omperazole-treated cells had 4-fold greater activity than controls.

In the last HH (not shown), the differences were not statistically different from control activity at any of the concentrations examined.

All three preparations showed statistically significant increases in CYP3A-mediated activity. Donor preparation HH993 showed approximately 8-fold induction at 10 μM R-95913 relative to controls, but activities returned toward controls at 100 μM. Concentration-dependent induction of up to about 6-fold was observed at all concentrations of R-95913 in preparation HH994. Concentration-related induction of up to about 3-fold was evident in preparation HH998 (not shown) at concentrations of 10 and 100 μM.

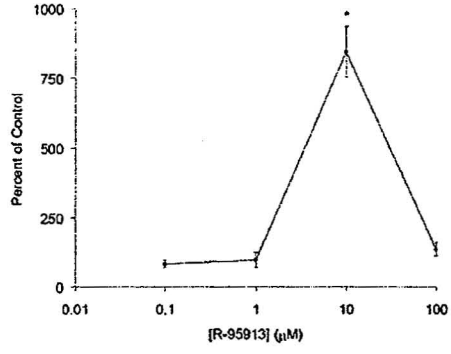

Figure 4: The effect of R-95913 on testosterone 6β-hydroxylase activity in human hepatocyte preparation HH993.

Activities are expressed as percent of control ± standard error of the mean. Mean control activity (n = 3) was 78.3 pmol/min/mg, and rifampicin-treated cells had 25-fold greater activity than that of control.

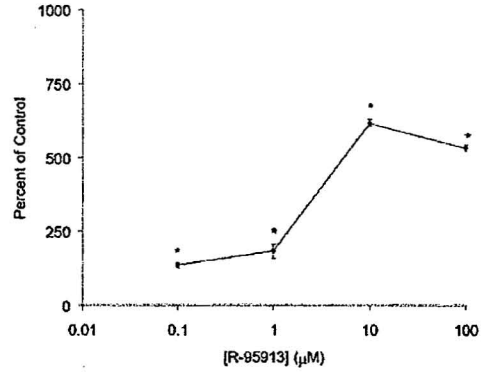

Figure 5: The effect of R-95913 on testosterone 6β-hydroxylase activity in human hepatocyte preparation HH994.

Activities are expressed as percent of control ± standard error of the mean. Mean control activity (n = 3) was 209 pmol/min/mg, and rifampicin-treated cells had 14-fold greater activity than that of control.

Comment:

This was a pilot study of the effects of catalytical activities for CYP1A2 and CYP3A in cultures of human hepatocytes. The results give some indication that the parent drug, prasugrel, may have a potential to induce hepatic CYP3A.

4.1.4 In Vitro Interaction of LY640315 (CS-747) Metabolites R-95913, R-138727, and R-106583 with Human Cytochrome P450 CYP3A

Study 2002IV-DI002
Conducted by: James A. Eckstein, Jessica L. Fayer, John B. Heim, Steve C. Kasper, Barbara J. Ring, and Steven A. Wrighton
Eli Lilly and Company Lilly Corporate Center Indianapolis, IN 46285
December 2002

Objective	To evaluate the abilities of the prasugrel active metabolite R-138727 and two inactive metabolites, R-95913 and R-106583, to inhibit metabolism mediated by CYP3A
Incubation Conditions Midazolam:	Time:1-minute Assay of 1'-hydroxy midazolam by LC/MS/MS. Human liver microsomes (0.1 mg protein) in 100 mM sodium phosphate buffer (pH 7.4), 1 mM NADPH, and midazolam (2.5 μM) in the absence or presence of R-138727 or R-106583 (0.5, 2.5, 10, 50, or 200 μM) or R-95913 (10, 20, 40, or 60 μM) midazolam: 2.5, 5.0, 10, 25, or 50 μM. Formation of 1'-hydroxy midazolam under these conditions was linear with respect to time.
Incubation Conditions Testosterone:	Time: 10 min Assay of 6ß-hydroxy testosterone by HPLC. R-138727 was not examined (co-elution of substances related to R-138727 with the 6ß-hydroxy testosterone). Human liver microsomes (0.075 mg protein) in 100 mM sodium phosphate buffer (pH 7.4), 1 mM NADPH, and testosterone (5.0 μM) in the absence or presence of R-106583 (0.5, 2.5, 10, 50, or 200 μM) or R-95913 (25, 100, 150, or 200 μM), and testosterone (5.0, 25, 50, 150, or 250 μM). Formation of 6ß-hydroxy testosterone under these conditions was linear with respect to time.
Data analysis	WinNonlin Professional, version 3.1 was used to estimate parameters: Km, Vmax, and Ki and the standard errors

Results

The formation of 1'-hydroxy midazolam by R-95913 was described with a competitive inhibition model with
Km 4.5 ± 0.7 μM
Vmax 921 ± 48 pmol/min/mg protein and
Ki 12.6 ± 2.2 μM.
There was ≤ 11% inhibition of CYP3A-mediated midazolam 1'-hydroxylation by R-138727 (see below)

Table 12. Effect of R-138727 In Vitro on the CYP3A Mediated Metabolism of Midazolam to 1'-Hydroxy Midazolam

Concentration of R-138727 (μM)	1'-Hydroxy Midazolam Formation (pmol/min/mg)	Percent of Control
0	348	100
0.5	343	99
2.5	338	97
10	368	106
50	334	96
200	311	89

and ≤ 18% inhibition of CYP3A-mediated midazolam 1'-hydroxylation by R-106583 (see below).

Table 13. Effect of R-106583 In Vitro on the CYP3A Mediated Metabolism of Midazolam to 1'-Hydroxy Midazolam

Concentration of R-106583 (μM)	1'-Hydroxy Midazolam Formation (pmol/min/mg)	Percent of Control
0	348	100
0.5	355	102
2.5	328	94
10	346	100
50	286	82
200	312	90

The formation of 6ß-hydroxy testosterone was described with a simple Michaelis-Menten kinetics by the human microsomal mixture in the inhibition study with R-95913, yielding an apparent Km value of 42.5 ± 5.0 μM and Vmax of 2259 ±88 pmol/min/mg protein.
The inhibition of testosterone metabolism by R-95913 was found to model best to noncompetitive inhibition yielding an apparent Ki value of 55.9 ± 4.2 μM. There was ≤ 24% inhibition of CYP3A-mediated testosterone 6ß-hydroxylase activity by R-106583 (see below).

Table 14. Effect of R-106583 In Vitro on the CYP3A Mediated Metabolism of Testosterone to 6ß-Hydroxy Testosterone

Concentration of R-106583 (μM)	6β-Hydroxy Testosterone Formation (pmol/min/mg)	Percent of Control
0	88.3	100
0.5	70.7	80
2.5	80.8	92
10	87.8	100
50	84.6	96
200	67.1	76

Comments:

1. The predicted in vivo inhibition by R-95913 of the CYP3A was calculated based on the highest reported mean Cmax value for R-95913 as 616 ng/mL (1.9 μM) following a 60 mg loading dose of prasugrel. An apparent Ki value for R-95913 was estimated