CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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STATISTICAL REVIEW(S)

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Science
Office of Biostatistics

Statistical Review and Evaluation

CARCINOGENICITY STUDIES

IND/NDA Number:	NDA 22-307
Drug Name:	CS-747 (Prasugrel)
Indication(s):	104 Week Carcinogenicity in Rats and Mice
Applicant:	Sponsor: Eli Lilly & Co., Indianapolis, IN Test Facility: Gotemba Laboratory, Bozo Research Center Inc. 1284 Kamado, Gotemba-shi, Shizuoka 412-0039, Japan
Documents Reviewed:	Electronic submission, Dated: Dec. 26, 2007 Electronic data submitted on Dec. 26, 2007
Review Priority:	Priority
Biometrics Division:	Division of Biometrics -6
Statistical Reviewer:	Mohammad Atiar Rahman, Ph.D.
Concurring Reviewer:	Karl Lin, Ph.D.
Medical Division:	Division of Cardiovascular and Renal Products
Reviewing Pharmacologist:	Belay Tesfamariam, Ph.D.
Project Manager:	Meg Pease-Fye
Keywords:	Carcinogenicity, Dose response

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1. Background

In this submission the sponsor included reports of two animal carcinogenicity studies, one in rats and one in mice. These studies were intended to assess the carcinogenic potential of CS-747 (Prasugrel) in rats and mice when administered orally by gavage at appropriate drug levels for about 104 weeks. Results of this review have been discussed with the reviewing pharmacologist Dr. Tesfamariam.

2. Rat Study

Two separate experiments were conducted, one in males and one in females. In each of these two experiments there were three treated groups and one control group. Two hundred and twenty F344/DuCrj (Fischer) SPF rats of each sex were randomly allocated to treated and control groups in equal size of 55 animals. The dose levels for treated groups were 10, 30, and 100 mg/kg/day. In this review these dose groups would be referred to as the low, medium, and high dose group, respectively. The controls received the vehicle (0.5w/v% tragacanth solution) by gavage.

During the administration period all animals were observed for physical and clinical signs three times everyday on normal week days and twice on weekends and holidays. In addition, palpation was performed once a week to detect superficial masses. A complete histopathological examination was performed on all animals from all groups found dead, killed moribund, or sacrificed during or at the end of the experiment.

2.1. Sponsor's analyses

2.1.1. Survival analysis

Survival function of each treatment group was estimated using the Kaplan-Meier product limit method. The dose response relationship[1] in mortality was tested using similar method as was suggested by Tarone. Pairwise comparisons of control and each treated group were performed using the Log-Rank test. All tests were conducted at one-tailed significance level of 0.05.

Sponsor's findings: Sponsor's analysis showed survival rates of 83.6%, 80.0%, 78.2%, and 89.1% in control, low, medium, and high dose groups, respectively in males and 69.1%, 72.7%, 78.2%, and 81.8%, respectively in females. Sponsor concluded that there was no statistically significant treatment related effect on the survival in either sex.

2.1.2. Tumor data analysis

Analysis for positive dose response relationship for tumor incidences among control, low, medium, and high dose groups and pairwise comparisons of control and treated groups were performed using the methods outlined in the paper of Peto et al. (1980). For incidental tumors, the analysis intervals were: weeks 0 - 52, 53 - 78, 79 - 92, and 93 till termination of the live phase. Exact permutation tests were used for tumors with less than 10 incidences.

Analysis for dose response relationship were conducted at the significance levels of 0.005 (one tailed-level) for common tumors and 0.025 (one tailed-level) for rare tumors. Pairwise comparison were conducted at the significance levels of 0.01 (one tailed-level) for common tumors and 0.05 (one tailed-level) for rare tumors.

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[1] In this review, the phrase "dose response relationship" refers to the linear component of the effect of treatment, and not necessarily to a strictly increasing or decreasing mortality or tumor rate as dose increases.

Common tumors were defined as those with a historical incidence in controls of 1% or more and rare tumors as less than 1%.

Reviewer's comment: *The above significance levels for dose response relationship test were suggested by Lin and Rahman (1998) and for pairwise comparisons were suggested by Haseman (1983) to adjust for multiple testing (to keep the false-positive rate at the nominal level of approximately 10%).*

Sponsor's findings: Sponsor's analyses showed no statistically significant positive dose response relationship or pairwise difference between control and any of the treated groups in any of the tested tumor types.

2.2. Reviewer's analyses

To verify sponsor's analyses and to perform additional analyses suggested by the reviewing pharmacologist, this reviewer independently performed survival and tumor data analyses. Data used in this reviewer's analyses were provided by the sponsor electronically.

2.2.1. Survival analysis

The survival distributions of animals in all four treatment groups were estimated by the Kaplan-Meier product limit method. The dose response relationship and homogeneity of survival distributions were tested using the Cox test (Cox, 1972) and the Generalized Wilcoxon test (Gehan, 1965). The intercurrent mortality data are given in Tables 1A and 1B in the appendix for males and females, respectively. The Kaplan-Meier curves for survival rate are given in Figures 1A and 1B in the appendix for males and females, respectively. Results of the tests for dose response relationship and homogeneity of survivals, are given in Tables 2A and 2B in the appendix for males and females, respectively.

Reviewer's findings: The tests showed no statistically significant dose response relationship or differences between the control and any of the treated groups in survivals across treatment groups in either sex.

2.2.2. Tumor data analysis

The tumor data were analyzed for dose response relationship and pairwise comparisons of control group with each of the treated groups were performed using the Poly-k method described in the paper of Bailer and Portier (1988) and Bieler and Williams (1993). One critical point for Poly-k test is the choice of the appropriate value of k. For long term 104 week standard rat and mouse studies, a value of k=3 is suggested in the literature. Hence, this reviewer used k=3 for the analysis of this data. For the calculation of p-values the exact permutation method was used. The tumor rates and the p-values of the tested tumor types are listed in Tables 3A and 3B in the appendix for males and females, respectively.

Multiple testing adjustment: Adjustment for the multiple dose response relationship testing was done using the results of Lin and Rahman (1998), which recommends the use of significance level of α=0.025 for rare tumors and of α=0.005 for common tumors for a submission with two studies, and a significance level of α=0.05 for rare tumors and of α=0.01 for common tumors for a submission with one study in order to keep the false-positive rate at the nominal level of approximately 10%. A rare tumor is defined as one in which the published spontaneous tumor rate is less than 1%. Adjustment for multiple pairwise comparisons was done using the results of Haseman (1983), which recommends the use of a significance level of α=0.05 for rare tumors and of α=0.01 for common tumors, in order to keep the false-positive rate at the nominal level of approximately 10%.

It should be noted that the recommended test levels by Lin and Rahman for the adjustment of multiple testing were originally based on the result of a simulation and an empirical study using the Peto method for dose response relationship analysis. However, some later simulation results by the same authors (unpublished manuscript presented in 2006 BASS meeting in Savanna, Georgia) indicated similar usefulness of their recommendation for Poly-3 analysis also.

Reviewer's findings: Following tumor types showed p-values less than or equal to 0.05 either for dose response relationship and/or pairwise comparisons of control and treated groups.

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pairwise Comparisons

Sex	Organ Name	Tumor Name	Cont N=55	Low N=55	Med N=55	High N=55	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
Male	Hemolymphoretic	LEUKEMIA,LARGE GRANU	8	8	3	2	0.009	0.500	0.100	0.026
	Mesothelium	MESOTHELIOMA,MALIGNA	4	3	1	1	0.047	0.358	0.181	0.100
	Prostate	ADENOMA	11	9	6	4	0.016	0.313	0.144	0.026
Female	Adrenal	PHEOCHROMOCYTOMA,MAL	2	0	0	0	0.031	0.248	0.121	0.121
	Hemolymphoretic	LEUKEMIA,LARGE GRANU	14	13	6	1	<0.0001	0.500	0.040	<0.0001
	Intestine,ileum	LEIOMYOSARCOMA	2	0	0	0	0.031	0.248	0.121	0.121

Based on the results of Lin and Rahman the incidence of none of the above or any other tested tumor types in either sex was considered to have statistically significant positive dose response relationship. Also based on the results of Haseman, none of the pairwise comparisons of treated groups with the control was considered to be statistically significant in either sex for increased tumor incidence in the treated group. A dose response relationship with negative slope was not considered to be statistically significant.

3. Mouse Study

Two separate experiments were conducted, one in males and one in females. In each of these two experiments there were three treated groups and one control group. Two hundred and twenty Crj:B6C3F$_1$ SPF mice of each sex were randomly allocated to treated and control groups in equal size of 55 animals. The dose levels for treated groups were 30, 100, and 300 mg/kg/day. In this review these dose groups would be referred to as the low, medium, and high dose group, respectively. The controls received the vehicle (0.5w/v% tragacanth solution) by gavage.

During the administration period all animals were observed for physical and clinical signs three times everyday on normal week days and twice on weekends and holidays. In addition, palpation was performed once a week to detect superficial masses. A complete histopathological examination was performed on all animals from all groups found dead, killed moribund, or sacrificed during or at the end of the experiment.

3.1. Sponsor's analyses

3.1.1. Survival analysis

Survival data from the mouse study were analyzed using the same statistical methodologies as were used to analyze the survival data from the rat study.

Sponsor's findings: Sponsor's analysis showed survival rates of 56.4%, 65.5%, 49.1%, and 49.1%, in control, low, medium, and high dose groups, respectively in males and 63.6%, 60.0%, 58.2%, and 56.4%, respectively in females. Sponsor concluded that there was no statistically significant treatment related effect on the survival in either sex.

3.1.2. Tumor data analysis

Tumor data from the mouse study were also analyzed using the same statistical methodologies as were used to analyze the tumor data from the rat study.

Sponsor's findings: Sponsor's analysis showed a statistically significant positive dose response relationship in the incidence of hepatocellular adenoma in both sexes. Pairwise comparisons showed statistically significant increased incidence of hepatocellular adenoma in high dose group of male and medium and high dose groups of females compared to their respective control.

3.2. Reviewer's analyses

This reviewer independently performed survival and tumor data analyses from the mouse study. For the mouse data analyses this reviewer used similar methodologies as he used to analyze the data from the rat study. Data used in this reviewer's analyses were provided by the sponsor electronically.

3.2.1. Survival analysis

The intercurrent mortality data are given in Tables 4A and 4B in the appendix for males and females, respectively. The Kaplan-Meier curves for death rate are given in Figures 2A and 2B in the appendix for males and females, respectively. Results for test of dose response relationship and homogeneity of survivals among treatment groups are given in Tables 5A and 5B in the appendix for males and females, respectively.

Reviewer's findings: The tests showed no statistically significant dose response relationship or differences between the control and any of the treated groups in survivals across treatment groups in either sex.

3.2.2. Tumor data analysis

The tumor rates and the p-values of the tumor types tested for dose response relationship and pairwise comparisons of control and treated groups are given in Table 6A and 6B in the appendix for males and females, respectively.

Reviewer's findings: Following tumor types showed p-values less than or equal to 0.05 either for dose response relationship or pairwise comparisons of control and treated groups.

Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pairwise Comparisons

Sex	Organ Name	Tumor Name	Cont Vehic N=55	Low 30mg N=55	Med 100mg N=55	High 300mg N=55	P_Value Dos Resp	P_Value C vs. L	P_Value C vs. M	P_Value C vs. H
Male	Liver	ADENOMA,HEPATOCELLUL	20	11	26	44	<0.0001	0.065	0.051	<0.0001
		ADENOMA+CARCINOMA	28	22	34	50	<0.0001	0.274	0.032	<0.0001
		HEMANGIOMA	6	3	1	1	0.016	0.244	0.058	0.058
	Spleen	HEMANGIOMA	4	0	1	0	0.014	0.059	0.183	0.060
Female	Liver	ADENOMA,HEPATOCELLUL	5	5	20	39	<0.0001	0.500	<0.0001	<0.0001
		ADENOMA+CARCINOMA	6	9	22	40	<0.0001	0.288	<0.0001	<0.0001
	Pituitary	ADENOMA,INTERMEDIATE	1	0	3	3	0.049	0.500	0.309	0.181
	Skin	SARCOMA,SPINDLE CELL	3	0	0	0	0.015	0.121	0.121	0.121

Based on the results of Lin and Rahman, the incidence of hepatocellular adenoma and combined incidences of hepatocellular adenoma and hepatocellular carcinoma in both sexes were considered to have statistically significant positive dose response relationships. Also based on the results of Haseman, the increased incidence of hepatocellular adenoma and combined incidences of hepatocellular adenoma and hepatocellular carcinoma in high dose group in males, and medium and high dose groups in females were considered to be statistically significant compared to their respective control. A dose response relationship with negative slope was not considered to be statistically significant.

4. Summary

In this submission the sponsor included reports of two animal carcinogenicity studies, one in rats and one in mice. These studies were intended to assess the carcinogenic potential of CS-747 (Prasugrel) in rats and mice when administered orally by gavage at appropriate drug levels for about 104 weeks.

In this review, the phrase "dose response relationship" refers to the linear component of the effect of treatment, and not necessarily to a strictly increasing or decreasing mortality or tumor rate as dose increases.

Rat Study: Two separate experiments were conducted, one in males and one in females. In each of these two experiments there were three treated groups and one control group. Two hundred and twenty F344/DuCrj (Fischer) SPF rats of each sex were randomly allocated to treated and control groups in equal size of 55 animals. The dose levels for treated groups were 10, 30, and 100 mg/kg/day. The controls received the vehicle (0.5w/v% tragacanth solution) by gavage. The tests showed no statistically significant dose response relationship or differences in survival across treatment groups in either sex. Tests did not show statistically

significant positive dose response relationship or increased incidence in treated group compared to the control in any of the tested tumor types.

Mouse Study: Two separate experiments were conducted, one in males and one in females. In each of these two experiments there were three treated groups and one control group. Two hundred and 20 Crj:B6C3F$_1$ SPF mice of each sex were randomly allocated to treated and control groups in equal size of 55 animals. The dose levels for treated groups were 30, 100, and 300 mg/kg/day. The controls received the vehicle (0.5w/v% tragacanth solution) by gavage. The tests showed no statistically significant dose response relationship or differences in survival across treatment groups in either sex. Tests showed statistically significant positive dose response relationship in the incidence of hepatocellular adenoma and combined incidences of hepatocellular adenoma and hepatocellular carcinoma in both sexes. Pairwise comparisons showed statistically significantly increased incidence of hepatocellular adenoma and combined incidences of hepatocellular adenoma and hepatocellular carcinoma in high dose group in males, and medium and high dose groups in females compared to their respective control.

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