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STATISTICAL REVIEW AND EVALUATION Clinical Studies

NDA/Serial Number: 21-882 / 000

Drug Name: ICL670/Exjade (deferasirox) (125, 250, 500 mg) Tablets for Oral Suspension

Indication(s): Treatment of chronic iron overload due to blood transfusions (transfusional hemosiderosis) in adult and pediatric patients as young as two years of age

Applicant: Novartis Pharmaceuticals Corporation

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1. EXECUTIVE SUMMARY

1.1 Conclusions and Recommendations

The one controlled study submitted does not provide clear statistical evidence supporting the non-inferiority of Exjade (deferiprison) to deferoxamine mesylate in lowering liver iron content, based on treatment success rate, in the treatment of chronic iron overload due to blood transfusions in patients with β -thalassemia major.

1.2 Background

The Sponsor has submitted one randomized, open-label, active comparator controlled, parallel group, multicenter, multinational study and one open-label, multicenter, multinational study to evaluate the efficacy and safety of Exjade (deferiprison) in the treatment of chronic iron overload due to blood transfusions (transfusional hemosiderosis). The design of the controlled study was developed with the Agency through a Special Protocol Assessment under IND 58,554 (Serial nos. 036, 037, and 039), as was the uncontrolled study. According to the Sponsor (Section 1, page 8 of protocol or page 7723 of submission):

Because humans are unable to actively eliminate iron from the body, toxic and eventually lethal levels of iron will accumulate as a result of repeated transfusions, e.g. in β -thalassemia major ... Excess iron is deposited in the form of hemosiderins (insoluble "iron cores" of ferritin) mainly in the liver, spleen, endocrine organs and in the myocardium. The exact mechanism of iron damage to these tissues is unknown, but it is established that organ failure correlates with iron burden in these tissues. Except for infectious diseases, cardiac complications are the major cause of death in patients with β -thalassemia major.

Iron chelators slowly mobilize these iron deposits, probably by a process of continuously binding the microscopic amounts of soluble iron present in the "transit pool", which are in equilibrium with the insoluble hemosiderins. Solubilized, chelated iron is excreted in the urine and/or via the bile into feces, the route of elimination in part depending upon the chemical structure of the chelating agent.

The current reference chelating agent in patients with transfusional hemosiderosis is deferoxamine mesylate (DFO) and it is the active comparator used in this study. Its route of administration, subcutaneous infusions lasting at least 8 hours at doses between 20 and 60 mg/kg/day given for 5 days/week, accounts for patients opting not to have treatment and for poor compliance among patients who are treated with it.

The Sponsor's proposed indication for Exjade (deferiprison), an iron chelator, is:

Exjade is indicated for the treatment of chronic iron overload due to blood transfusions (transfusional hemosiderosis) in adult and pediatric patients as young as two years of age.

1.3 Statistical Issues and Findings

Statistical issues are failure in the protocol-specified primary efficacy analysis and the assessment of strength of evidence in secondary endpoints and subgroup analyses. Subgroup analyses are presented and discussed in Section 3.

The following is the result of the analysis of the primary efficacy endpoint in patients with β -thalassemia major:

- Study C1670A0107 does not demonstrate the non-inferiority of ICL670 to DFO in terms of treatment success in lowering liver iron content (lower bound of 95% confidence interval for difference in change from baseline for treatment success is not greater than -0.15 is the primary endpoint analysis).

2. INTRODUCTION

2.1 Overview

The Sponsor has submitted the following two studies:

- A controlled Phase 3 efficacy clinical study in male and female subjects at least 2 years of age with β -thalassemia and transfusional hemosiderosis and a liver iron content of at least 2mg/g dw and previously treated with deferoxamine (DFO) or never treated with any iron chelator
- An uncontrolled Phase 2 efficacy clinical study in male and female subjects at least 2 years of age with rare chronic anemias other than β -thalassemia and β -thalassemia and transfusional hemosiderosis and a liver iron content of at least 2mg/g dw and unable to be treated with DFO.

The controlled study is designed to assess the efficacy, in terms of non-inferiority, of ICL670 in the treatment of chronic iron overload (transfusional hemosiderosis) compared to the reference chelating agent, DFO, in patients with transfusional hemosiderosis. The target patient population of the Phase 3 study consists of 500 β -thalassemia outpatients with transfusional hemosiderosis and previously treated with DFO, randomized 1:1 to one of two groups of 250 patients each.

The uncontrolled study is designed to assess the efficacy of ICL670, in terms of success rate, in the treatment of chronic iron overload in patients with transfusional hemosiderosis. The target patient population of the Phase 2 study consists of 175 patients, of which 100 are outpatients with rare chronic anemias other than β -thalassemia (stratum 1) and 75 outpatients with β -thalassemia (stratum 2) with transfusional hemosiderosis unable to be treated with DFO. Table 2.1 presents a brief summary of the two studies addressed in this review.

Table 2.1
Brief Summary of Clinical Studies for Exjade

Study Number (No. and Location of Centers) and Dates of Study Conduct	Subject Population	Treatment	Number Randomized (Treated)	Design ¹
CICL670A0107 (Phase 3) (46 Europe, 3 N. Africa, 5 S. America, 2 Canada, 7 U.S.) 2-13-03 to 11-2-04	Male and female patients with β -thalassemia and transfusional hemosiderosis and a liver iron content of ≥ 2 mg Fe/g dry weight, at least 2 yrs. of age	Exjade (ICL670) Deferoxamine (DFO) Total	297 (296) 294 (290) 591 (586)	OL, R, AC, PG, MC
CICL670A0108 (Phase 2) (29 Europe, 3 Canada, 6 U.S.) 1-27-03 to 11-5-04	Male and female patients with β -thalassemia, congenital or acquired chronic anemia, and transfusional hemosiderosis and a liver iron content of ≥ 2 mg Fe/g dry weight, at least 2 yrs. of age	Exjade (ICL670) Total	184 (184) 184 (184)	OL, MC

Source: Statistical Reviewer's listing.

¹ OL = Open Label, R = Randomized, AC = Active Comparator, PG = Parallel Group, MC = Multicenter

The Sponsor's proposed indication for Exjade (deferiasirox), an iron chelator, is:

Exjade is indicated for the treatment of chronic iron overload due to blood transfusions (transfusional hemosiderosis) in adult and pediatric patients as young as two years of age.

My review presents the Sponsor's protocol-specified primary efficacy analyses for success of lowering LIC in detail and briefly presents clinically relevant secondary efficacy analyses.

2.2 Data Sources

The study reports and additional information for these studies were submitted electronically. The submitted SAS data sets for all studies were complete and well documented. These items were located in the Electronic Document Room at \\Cdsub1\N21882\N 000\2005-04-29.

3. STATISTICAL EVALUATION

3.1 Evaluation of Efficacy

3.1.1 Study Design

3.1.1.1 Phase 3 Study CICL670A0107

In this study, screened eligible subjects went through a 5-day wash-out period before randomization to treatment. After randomization, the subject had visits every 4 weeks until end of study (1 year) or discontinuation. Subjects were randomized in a 1:1 ratio to receive ICL670 or Deferoxamine mesylate (Desferal- DFO), the active comparator and reference therapy.

The initial dose selected at start of study treatment depended on the LIC determined at screening. ICL670 is administered as an oral dose between 5 and 30 mg/kg/day. DFO is administered as subcutaneous infusions lasting at least 8 hours at doses between 20 and 60 mg/kg/day given for 5 days/week. Patients with a baseline LIC between 2 and 7 mg Fe/g dw were allowed to continue their previous doses and schedules of DFO treatment, even if not meeting the requirements given in Table 3.1 below. Table 3.1 gives the dose administered depending on the value

of the screening LIC. The initial dose is fixed during the one-year study period, unless safety concerns, e.g. overchelation, during the study support a dose adjustment.

Table 3.1
Study C1CL670A0107: Initial Dose of Study Treatment Based on Screening Liver Iron Content (LIC)

Screening LIC	Initial ICL670 Dose	Initial DFO Dose
2 to 3 mg Fe/g dw	5 mg/kg/day	20 - 30 mg/kg/day
> 3 to 7 mg Fe/g dw	10 mg/kg/day	25 - 35 mg/kg/day
> 7 to 14 mg Fe/g dw	20 mg/kg/day	35 - 50 mg/kg/day
> 14 mg Fe/g dw	30 mg/kg/day	≥ 50 mg/kg/day

Source: Statistical Reviewer listing.

All patients underwent a baseline liver biopsy that was repeated one year later. Liver biopsy was the reference technique for standardized determination of LIC in all patients (in pediatric patients, where liver biopsy was not practicable, the use of SQUID was discussed with the Sponsor on a case by case basis). Biopsy analysis was performed at one pathology laboratory. The Sponsor stated that (Section 3.2, page 15 of protocol or page 7730 of submission):

SQUID is a new technology for the non-invasive determination of liver iron, based on the measurement of the magnetic susceptibility of the liver, and has been shown to be accurate and provide results that reproducibly correlate with those achieved by standard methods of iron determination in liver biopsy samples.

The Clinical Reviewer investigated this claim and determined that the Sponsor's conclusions were based on one article that utilized "First preliminary results in phantoms and in 4 patients" (Starr TN, et.al. A new generation SQUID biosusceptometer, Nenonen J, Ilmoniemi RJ, Katila T (eds): Biomag 2000: Proceedings 12th Int Conf Biomagnetism, Section 3.3).

The Sponsor stated in the study report (Section 9.1, page 78) that:

LIC measured by SQUID consistently gave lower results than when measured by liver biopsy. Of the 90 SQUID evaluations at baseline in the PP-1 population, 64 (71.1%) gave LIC values <7 mg Fe/g dw in comparison to only 108/463 (23.3%) liver biopsies. On average, LIC values measured by SQUID were about 50% of those measured by biopsy.

As a consequence, patients with baseline SQUID assessments were more likely to have a LIC <7 mg Fe/g dw and should therefore have received lower doses of study drug when compared to patients undergoing liver biopsy. However, this situation preferentially affected patients randomized to ICL670 because the protocol allowed patients randomized to DFO to continue on their previous dose if baseline LIC was <7 mg Fe/g dw whereas patients randomized to ICL670 were dosed strictly according to the protocol. This inherent dosing bias flawed the comparison of ICL670 and DFO patients in the PP-1 population with LIC <7 mg Fe/g dw. This bias did not occur in the two higher dose cohorts since patients with LIC ≥7 mg Fe/g dw had to take both DFO and ICL670 according to baseline LIC.

Given that SQUID underestimates LIC and is not a validated method to determine liver iron content (per the Clinical Reviewer), its utility as a method to provide (useful, valid, quality) data for efficacy analysis has not been determined. As described in Section 3.1.3, the above issues with SQUID prompted the Clinical Reviewer to request additional subgroup analyses to describe efficacy.

3.1.1.2 Phase 2 Study C1CL670A0108

The design of study C1CL670A0108 is similar to that of study C1CL670A0107, as described above, except in the following two aspects:

- It is a Phase 2, uncontrolled study, without DFO as a comparator
- SQUID LIC assessment was performed in patients with a medical contraindication to liver biopsy (such as coagulation problems in patients with myelodysplastic syndrome), as determined on a case by case basis with the Sponsor.

The same problems with SQUID, as described above, are also relevant in this study.

3.1.2 Primary Efficacy Analysis

3.1.2.1 Phase 3 Study C1670A0107

The primary objective of study C1670A0107 is to demonstrate the non-inferiority of ICL670 to DFO in terms of effects on LIC assessed by liver biopsy after one-year treatment in β -thalassemia patients with transfusional hemosiderosis. In some cases in the pediatric population, where liver biopsy was not practicable SQUID alone was used to assess LIC. For patients where SQUID and liver biopsy was available, the biopsy data was used for the primary efficacy analysis.

The protocol-specified primary analysis was based on success rate, defined as a binary outcome indicating a successful or failed treatment effect on LIC, and analyzed in terms of non-inferiority. As presented in Table 3.2, depending on the LIC at baseline, different criteria had to be met to achieve a treatment success or failure. Non-inferiority was to be demonstrated if the lower bound of the two-sided 95% confidence interval (CI) of the difference in success rate (ICL670 - DFO) was greater than -0.15. The CI is estimated based on asymptotic normal approximation for the difference of two binomial probabilities.

Table 3.2
Study C1670A0107: Success Criteria for Primary Efficacy Evaluation

LIC at Baseline	Success, if LIC is	Failure, if LIC is
2 to < 7 mg Fe/g dw	1 to < 7 mg Fe/g dw	< 1 mg Fe/g dw
≥ 7 to < 10 mg Fe/g dw	1 to < 7 mg Fe/g dw	≥ 7 mg Fe/g dw
≥ 10 mg Fe/g dw	Decrease in LIC ≥ 3 mg Fe/g dw	< 1 mg Fe/g dw
		≥ 7 mg Fe/g dw
		Decrease in LIC < 3 mg Fe/g dw

Source: Statistical Reviewer's listing.

The primary efficacy analysis population was the protocol-specified per protocol (PP-1). The PP-1 population included patients that received study drug and who had an LIC determination at baseline and at end of study, using the same technique as at baseline. Also included were patients who had to permanently discontinue study medication due to any of the following safety reasons: adverse event, abnormal laboratory value, abnormal test procedure results, or iron overload related death. Subjects with no LIC result available at one year using the same technique as at baseline were counted as treatment failures in the PP-1 population. Patients who discontinued study drug for the safety reasons indicated in the PP-1 definition were considered treatment failures, since the treatment was obviously not tolerated and the patient could not benefit from the drug. Patients prematurely discontinued due to any other reason were excluded from this population.

3.1.2.2 Phase 2 Study C1670A0108

The primary objective of Phase 2 study C1670A0108 is to demonstrate a pre-specified success rate for ICL670 in terms of effects on LIC assess by liver biopsy after one-year treatment in outpatients with rare chronic anemias other than β -thalassemia and outpatients with β -thalassemia with transfusional hemosiderosis unable to be treated with DFO. In some patients with a medical contraindication to liver biopsy, SQUID alone was used to assess LIC.

The protocol-specified primary analysis was based on success rate, defined as a binary outcome indicating a successful or failed treatment effect on LIC, and analyzed in terms of comparison to a pre-specified threshold. As presented in Table 3.2, depending on the LIC at baseline, different criteria had to be met to achieve a treatment success or failure. In order to claim that the success rate of ICL670 is greater than 50%, a test statistic based on the standardized normal approximation of the binomial distribution was used at one-sided alpha level of 0.025.

The primary efficacy analysis population was the ITT population, which included all patients that successfully passed screening and were chosen to start study treatment. In addition, an analysis using the PP-1 population was planned to be reported. Subjects with no LIC result available at one year using the same technique as at baseline, were counted as treatment failures in the ITT and PP-1 populations.

For hypothesis testing in the ITT and PP-1 populations, data from both strata, β -thalassemia and rare anemias, were pooled. No separate hypothesis testing was planned to be performed per stratum, but estimates of the success rate and corresponding confidence intervals were calculated for both strata.

3.1.3 Important Secondary Efficacy and Subgroup Analyses

3.1.3.1 Phase 3 Study C1CL670A0107

The Clinical Reviewer requested that an important protocol-specified secondary endpoint analysis, the comparison of the change in LIC value from baseline between patients treated with ICL670 and DFO for patients that had a LIC of ≥ 7 mg Fe/ g dw at baseline, be reviewed. The protocol-specified analysis was the fitting of an analysis of covariance model (ANCOVA) with baseline LIC value as the covariate and treatment group as the main effect. A between treatment comparison was performed using the least square means obtained from the model, with the least square estimated difference, two-sided 95% confidence interval, and p-value presented.

In addition to the important secondary efficacy endpoint analysis, the Clinical Reviewer also requested that the following *post hoc* subgroup analyses of the primary and important secondary efficacy endpoint listed in Table 3.3 be presented to investigate efficacy in these subgroups. These 14 analyses, presented by the Sponsor, consist of 2 protocol-specified and 12 *post hoc* analyses. The following are some clinical reasons for these subgroup analyses:

- SQUID is not a validated technique for measuring LIC and underestimates the LIC by at least 50%.
- The lower initial doses of ICL670 for those with baseline LIC < 7 mg Fe/g dw may not be efficacious.
- The effect of inaccurate SQUID LIC values resulted in patients being underdosed with ICL670.

Table 3.3
Study C1CL670A0107: Listing of Important Protocol-Specified Secondary Endpoint Analysis and
***Post Hoc* Subgroup Analyses of the Primary Efficacy Endpoint and Important Secondary Endpoint**

Endpoint Type	Endpoint Description	Subgroup Analysis	Number of Analyses	Protocol-Specified or <i>Post Hoc</i> Analysis
Primary	Treatment success rate	Biopsy	1	<i>Post hoc</i>
		SQUID	1	<i>Post hoc</i>
		Baseline LIC group*	2	<i>Post hoc</i>
		Biopsy X Baseline LIC group*	2	<i>Post hoc</i>
		SQUID X Baseline LIC group*	2	<i>Post hoc</i>
Secondary	LIC value change from baseline	Baseline LIC < 7 mg Fe/g dw	1	<i>Post hoc</i>
		Baseline LIC ≥ 7 mg Fe/g dw	1	Protocol-specified
		Biopsy X Baseline LIC group*	2	<i>Post hoc</i>
		SQUID X Baseline LIC group*	2	<i>Post hoc</i>

Source: Statistical Reviewer listing.

* The two baseline LIC groups are as follow: < 7 mg Fe/g dw and ≥ 7 mg Fe/g dw.

All secondary efficacy analyses utilized another protocol-specified per population, the PP-2 population, which included patients who had an end of study LIC value, using the same technique as at baseline. Also, the above listed secondary and *post hoc* analyses were not taken into account for powering the study, so the results are exploratory.

3.1.3.2 Phase 2 Study C1CL670A0108

The Clinical Reviewer requested that the following three *post hoc* analyses presented in Table 3.4 be reviewed. Descriptive statistics of success rate and its 95% confidence interval are presented.

Table 3.4
Study C1CL670A0108: Listing of *Post Hoc* Subgroup Analyses of the Primary Efficacy Endpoint

Endpoint Description	Subgroup Analysis	Protocol-Specified or <i>Post Hoc</i> Analysis
Treatment success rate	Biopsy patients in PP-1 population	<i>Post hoc</i>
	Biopsy patients with baseline LIC ≥ 7 mg Fe/g dw in ITT population	<i>Post hoc</i>
	β -thalassemia biopsy patients with baseline LIC ≥ 7 mg Fe/g dw in PP-1 population	<i>Post hoc</i>

Source: Statistical Reviewer listing.

This review presents the protocol-specified and *post hoc* primary efficacy analyses and briefly presents the important secondary efficacy analyses, both protocol-specified and *post hoc* for both studies.

3.1.4 Phase 3 Study C1CL670A0107 Results

3.1.4.1 Overall Study Descriptive Information

The following section presents demographic and baseline characteristics, subject disposition, and distribution of liver iron content measurement techniques.

Demographic and baseline characteristics are comparable among the treatment groups in the treated population. The subjects' mean age is 17 years for ICL670 and 17.3 years for DFO. The majority of subjects are Caucasian (88.9% for ICL670 and 86.6% for DFO) and female (52.7% for ICL670 and 51% for DFO).

Table 3.5 presents the number of randomized subjects and their disposition. Subjects were randomized in a 1:1 fashion to each treatment group as specified in the protocol. After randomization, but before starting study treatment, five patients (one ICL670, four DFO) withdrew consent. So, 296 patients on ICL670 and 290 patients on DFO began therapy. Study discontinuation is 5.7% in the ICL670 group and 4.1% in the DFO group. The primary reasons for study discontinuation in the ICL670 group are adverse events and withdrawal of consent, and in DFO group are withdrawal of consent and death. Overall, the subjects were balanced in the PP-1 and PP-2 analysis population groups.

Table 3.5
Summary of Subject Disposition and Analysis Populations for Controlled Study C1CL670A0107

	ICL670	DFO
Randomized (ITT)	297	294
Treated	296 (99.7)	290 (98.6)
Completed Treatment*	279 (94.3)	278 (95.9)
Discontinued Treatment* n (%)	17 (5.7)	12 (4.1)
Discontinued Due to Adverse Events** n (%)	7 (41.2)	1 (8.3)
Death	1 (5.9)	3 (25.0)
Protocol Violation	2 (11.8)	2 (16.7)
Withdrawal of Consent	7 (41.2)	6 (50.0)
Per Protocol Population 1 (PP-1)* n (%)	276 (93.2)	277 (95.5)
Per Protocol Population 2 (PP-2)* n (%)	268 (90.5)	273 (94.1)

Source: Table 7-1, page 62, Study ICL670A0107 report.

* With respect to number of treated subjects.

** With respect to number of all discontinuations.

In the per protocol population 1 (PP-1), the majority of LIC measurements were made using biopsy (229/276 = 83.0% for ICL670 and 234/277 = 84.5% for DFO) compared to SQUID.

3.1.4.2 Primary Efficacy Results

The Sponsor's results for the protocol-specified primary efficacy analysis of non-inferiority and *post hoc* subgroup analyses requested by the Clinical Reviewer are presented in Table 3.6. The protocol-specified primary efficacy analysis is presented in the shaded area. Non-inferiority of ICL670 to DFO has not been demonstrated because the lower bound of the 2-sided 95% confidence interval for the difference in treatment success rate (ICL670 - DFO) is greater than -0.15.

Results for the subgroup analyses are as follow:

- For all patients, the lower bound of the 95% C.I. for the difference in treatment success rate was not greater than -0.15 for either the biopsy or SQUID subgroup.
- For those patients with a baseline LIC < 7mg Fe/g dw, the lower bound of the 95% C.I. for the difference in treatment success rate was not greater than -0.15 for either the biopsy & SQUID, biopsy or SQUID subgroup.
- For those patients with a baseline LIC ≥ 7mg Fe/g dw, the lower bound of the 95% C.I. for the difference in treatment success rate was greater than -0.15 for the biopsy & SQUID and biopsy subgroups but not for the SQUID subgroup.

Table 3.6
Study ICLOA0107: Success Rates Based on Change in Liver Iron Content (PP-1 population) for ICL670 and DFO

	Overall		LIC < 7 mg Fe/g dw		LIC ≥ 7 mg Fe/g dw	
LIC Measurement Technique	ICL670	DFO	ICL670	DFO	ICL670	DFO
Biopsy & SQUID[†]						
N	276	277	85	87	191	190
Success Rate (n)	52.9 (146)	66.4 (184)	40.0 (34)	82.8 (72)	58.6 (112)	58.9 (112)
Difference and 95% C.I. *	-13.5 [-21.6, -5.4]		-42.8 [-55.9, -29.7]		-0.3 [-10.2, 9.6]**	
Biopsy						
N	229	234	53	55	176	179
Success Rate (n)	51.1 (117)	62.8 (147)	22.6 (12)	76.4 (42)	59.7 (105)	58.7 (105)
Difference and 95% C.I. *	-11.7 [-20.7, -2.8]		-53.7 [-69.6, -37.8]		-1.0 [-9.2, 11.2]**	
SQUID						
N	47	43	32	32	15	11
Success Rate (n)	61.7 (29)	86.0 (37)	68.8 (22)	93.8 (30)	46.7 (7)	63.6 (7)

Source: Table 9-1, page 77, ICLOA0107 Study Report.

[†] Primary efficacy analysis.

* The two-sided, 95% confidence interval is based on an asymptotic normal approximation for the difference of two binomial probabilities.

** The lower bound of the 95% confidence interval is greater than the -0.15 non-inferiority bound.

3.1.4.3 Secondary Efficacy Results

The protocol-specified secondary analysis is presented in the shaded area of Table 3.7 below. The other secondary analyses are *post hoc*, presented by the Sponsor, and what the Clinical Reviewer requested be presented.

The result of the protocol-specified secondary analysis is as follows:

- In patients with a baseline LIC ≥ 7 mg Fe/g dw, there was no difference in the change from baseline in LIC between the ICL670 and DFO treatment groups (95% C.I. is from -1.79 to 0.66).

Results of the *post hoc* secondary analyses are as follow:

- In patients with a baseline LIC < 7 mg Fe/g dw, all subgroups (biopsy & SQUID, biopsy alone, SQUID alone) there was an increase in the change from baseline in LIC between the ICL670 and DFO treatment groups. That is, there was a greater increase in LIC from baseline for ICL670 than for DFO (the lower bounds of all 95% C.I.s. are >0.0).
- In patients with a baseline LIC ≥ 7 mg Fe/g dw, for the biopsy alone and SQUID alone subgroups, there was no difference in the change from baseline in LIC between the ICL670 and DFO treatment groups (both 95% C.I.s include zero).

Table 3.7
Study C1CL0A0107: Change from Baseline in Liver Iron Content between Treatments by Baseline Liver Iron Content Category (PP-2 population)

LIC Measurement Technique	LIC < 7 mg Fe/g dw			LIC ≥ 7 mg Fe/g dw		
	ICL670	DFO	Difference	ICL670	DFO	Difference
Biopsy & SQUID						
n	83	87		185	186	
Mean (s.e.)*	3.99 (0.34)	0.15 (0.33)	3.84 (0.48)	-5.08 (0.44)	-4.52 (0.44)	-0.56 (0.62)
95% C.I.*	[3.32, 4.66]	[-0.50, 0.80]	[2.90, 4.77]	[-5.94, -4.22]	[-5.38, -3.66]	[-1.79, 0.66]
Biopsy						
n	52	55		172	175	
Mean (s.e.)*	5.54 (0.44)	0.54 (0.43)	5.00 (0.62)	-5.31 (0.47)	-4.66 (0.46)	-0.65 (0.66)
95% C.I.*	[4.68, 6.40]	[-0.30, 1.38]	[3.77, 6.22]	[-6.23, -4.39]	[-5.56, -3.76]	[-1.95, 0.65]
SQUID						
n	31	32		13	11	
Mean (s.e.)*	1.40 (0.31)	-0.52 (0.31)	1.92 (0.44)	-1.62 (0.89)	-2.81 (0.98)	1.19 (1.34)
95% C.I.*	[0.79, 2.01]	[-1.13, 0.09]	[1.04, 2.80]	[-3.36, 0.12]	[-4.73, -0.89]	[-1.60, 3.98]

Source: Post-text Table 9.2-16, pages 3570 and 3571, ICL0A0107 Study Report and Statistical Reviewer analyses.

* Mean, standard error, and 95% confidence interval based on an ANCOVA model with baseline LIC as covariate.

3.1.5 Phase 2 Study C1CL670A0108 Results

3.1.5.1 Overall Study Descriptive Information

The following section presents demographic and baseline characteristics, subject disposition, and distribution of liver iron content measurement techniques.

The subjects' mean age is 35 years, the majority of subjects are Caucasian (78.8%), and equal representation of male and female subjects (50%).

Table 3.8 presents the number of enrolled subjects and their disposition. All 184 enrolled patients began ICL670 therapy. Study discontinuation is 17.4%, with the primary reasons for study discontinuation being adverse events and withdrawal of consent.

Table 3.8
Summary of Subject Disposition and Analysis Populations for Uncontrolled Study C1CL670A0108

	ICL670
Enrolled (ITT)	184
Treated	184 (100.0)
Completed Treatment*	152 (82.6)
Discontinued Treatment* n (%)	32 (17.4)
Discontinued Due to Adverse Events** n (%)	13 (40.6)
Death	5 (15.6)
Study Drug No Longer Required	4 (12.5)
Withdrawal of Consent	10 (31.3)
Per Protocol Population 1 (PP-1)* n (%)	165 (89.7)

Source: Table 7-1, page 62, Study ICL670A0107 report and Table 7-1, page 55, Study ICL670A0108 report.

* With respect to number of treated subjects.

** With respect to number of all discontinuations.

Of the 184 enrolled (ITT) patients in Study C1CL670A0108, 85 had β -thalassemia and 99 had other rare anemias. Of the 165 patients in the PP-1 population, 80 had β -thalassemia and 85 had other rare anemias.

In the ITT population, the majority of LIC measurements were made using biopsy (120/184 = 65.2%) compared to SQUID.

3.1.5.2 Primary Efficacy Results

The Sponsor's results for the protocol-specified primary efficacy analysis of success rate and additional protocol-specified analyses of success rate are presented in Table 3.9. The protocol-specified primary efficacy analysis is presented in the shaded area. The study failed to show that the lower bound of the 95% confidence interval for the true success rate was greater than 50%.

Results for the additional protocol-specified analyses of success rate are as follow:

- For all patients in the PP-1 population, the study failed to show that the lower bound of the 95% confidence interval for the true success rate was greater than 50%.
- In β -thalassemia patients in the PP-1 population, the 95% confidence interval for success rate is [45.4, 67.1].
- In rare anemia patients in the PP-1 population, the 95% confidence interval for success rate is [45.9, 67.0].

Table 3.9
Study CICL0A0108: Success Rates Based on Liver Iron Content for ICL670

LIC Measurement Technique	ITT Population		PP-1 Population	
	All Patients	All Patients	β -thalassemia	Rare Anemias
Biopsy & SQUID[†]				
N	184	165	80	85
Success Rate (n)	50.5 (93)	56.4 (93)	56.3 (45)	56.5 (48)
95% C.I.*	[43.3, 57.8]	[48.8, 63.9]	[45.4, 67.1]	[45.9, 67.0]
p-value (1-sided, $\alpha=0.25$)	0.441	0.051		

Source: Table 9-1, page 75, ICL0A0108 Study Report.

[†] Primary efficacy analysis.

* The two-sided, 95% confidence interval is based on an asymptotic normal approximation for the binomial distribution.

3.1.5.3 Secondary Efficacy Results

The three *post hoc* descriptive analyses, presented by the Sponsor and what the Clinical Reviewer requested be reviewed, are presented in Table 3.7. Results of the *post hoc* descriptive analyses are as follow:

- In biopsy patients in the PP-1 population, the 95% confidence interval is [51.8, 70.0].
- In biopsy patients with a baseline LIC ≥ 7 mg Fe/g dw in the ITT population, the 95% confidence interval is [50.2, 68.4].
- In β -thalassemia patients with a baseline LIC ≥ 7 mg Fe/g dw in the PP-1 population, the 95% confidence interval is [55.2, 79.3].

Table 3.10
Study CICL0A0108: Success Rates Based on Liver Iron Content in Patients with Biopsy for ICL670

LIC Measurement Technique	All Patients in PP-1 Population	All Patients with LIC ≥ 7 mg Fe/g dw in ITT Population	β -thalassemia Patients with LIC ≥ 7 mg Fe/g dw in PP-1 Population
Biopsy			
N	110	113	58
Success Rate (n)	60.9 (67)	59.3 (67)	67.2 (39)
95% C.I.	[51.8, 70.0]	[50.2, 68.4]	[55.2, 79.3]

Source: Table 9-1, page 75, ICL0A0108 Study Report.

* The two-sided, 95% confidence interval is based on an asymptotic normal approximation for the binomial distribution.

3.2 Evaluation of Safety

There is no statistical evaluation of safety necessary for this review. For information, reference the clinical review evaluation of safety section.

4. FINDINGS IN SUBGROUP POPULATIONS

4.1 Gender, Race, and Age

The Sponsor has presented efficacy analyses by gender and age. There are no clinically important differences among groups defined by values of these variables. Of greater interest are findings by the subgroups described in section 4.2 below.

4.2 Other Special/Subgroup Populations

The subgroup populations of interest are listed in Tables 3.3 and 3.4 and addressed in Sections 3.1.4.3 and 3.1.5.3.

5. CONCLUSIONS

Study C1CL670A0107 does not provide clear and substantial statistical evidence demonstrating the non-inferiority of Exjade (deferasirox) to deferoxamine mesylate in lowering liver iron content in patients with β -thalassemia major undergoing treatment for chronic iron overload.

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this page is the manifestation of the electronic signature.**

/s/

Sonia Castillo
10/12/2005 01:18:39 PM
BIOMETRICS

Mike Welch
10/12/2005 01:40:32 PM
BIOMETRICS
Concur with review.

S. Edward Nevius
10/12/2005 05:21:24 PM
BIOMETRICS
Concur with review.



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

Statistical Review and Evaluation

CARCINOGENICITY STUDY ADDENDUM

BLA/Serial Number: NDA 21-882
Drug Name: Exjade (desferasirox)
Indication(s):
Applicant: Novartis
Date(s): Applicant's letter date: August 15, 2005
Review Priority: Standard
Biometrics Division: Biometrics Division 2
Statistical Reviewer: Ted Guo, Ph.D. (HFD-715)
Concurring Reviewers: Karl Lin, Ph.D., Biometrics Division 2
Medical Division: Gastrointestinal and Coagulation Drug Product Division (HFD-180)
Pharmacologist: Tamal Chakraborti, Ph.D., Pharmacologist (ODE 3, HFD-180)
Jasti B. Choudary, Ph.D., Supervisory Pharmacologist (ODE 3, HFD-180)
Project Manager: Alice Kacuba (ODE 3, HFD-180)
Keywords: NDA review, Carcinogenicity

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EXECUTIVE SUMMARY

The analyses of carcinogenic potential in male and female rats did not show a positive linear dose-tumor trend when the non-iron-diet control group was excluded.

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OVERVIEW OF CARCINOGENICITY STUDIES

The purpose of the submitted carcinogenicity study was to “determine the potential carcinogenicity profile of ICL670, an iron chelator, when administered orally to rats fed an iron supplemented diet for at least 104 weeks (p. 11, 017022.pdf).”

The dosage assignments to the rats are shown in the following table.

Table 1 ICL670 Dosage assignments to all rats (standard and iron diets)

Species	Sex	No. Animals per group	Dosage (mg/kg/day)
Rat	Male	50	0(s), 0, 15, 30, 60
	Female	50	0(s), 0, 15, 30, 60

Note that the dosage symbolized by 0(s) represents the feed in standard diet, i.e., with no ICL670 in the feed.

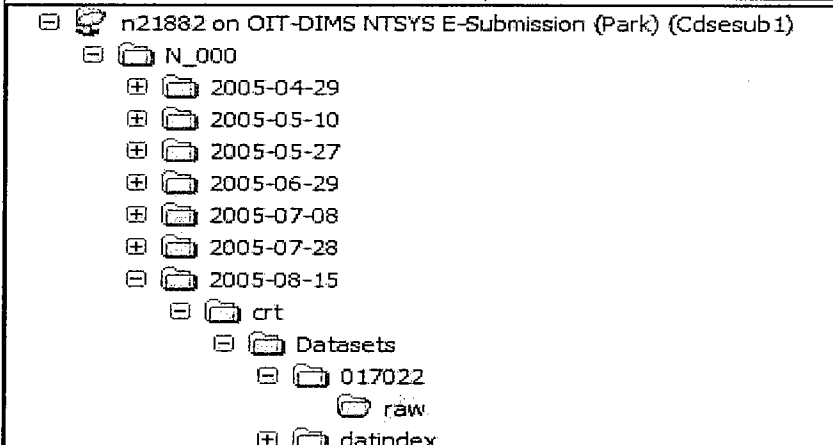
The sponsor concluded, “based on the results of this study, the oral administration of ICL670 via gavage to rats fed an iron supplemented diet for at least 104 weeks was not carcinogenic at doses up to 100>60 mg/kg/day (p. 11, 017022.pdf).”

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REVIEWER'S EVALUATION OF CARCINOGENICITY STUDIES

The statistical review is based on the sponsor's data submitted on August 15, 2005 upon request from the Agency on June 23, 2005. One data file, TUMOR.XPT, was received by the Agency. The study report was named **Study no. 017022, 104-week oral (gavage) carcinogenicity study in rats.**

Table 2 Definition files for the tumor data set

Novartis Study - No. 017022		Compound: ICL670
A 104-week oral carcinogenicity study in rats		
Confidential		23-June-2005
DATA DEFINITION TABLES		
Datasets for Study 017022		
Dataset	Description of Dataset	Location of dataset
Tumor	Tumor frequency for statistical analysis	tumor.xpt
		

Source: crtloc.pdf

The sponsor's data have been regulated as a common practice in the statistical review. Not an original data value has been altered or changed. The analysis data sets are listed in the following table.

Table 3 Analysis data files

Name	Explanation
R2M21882	Male-rat data
R2F21882	Female-rat data

Analysis of Male Rats

Mortality Analysis

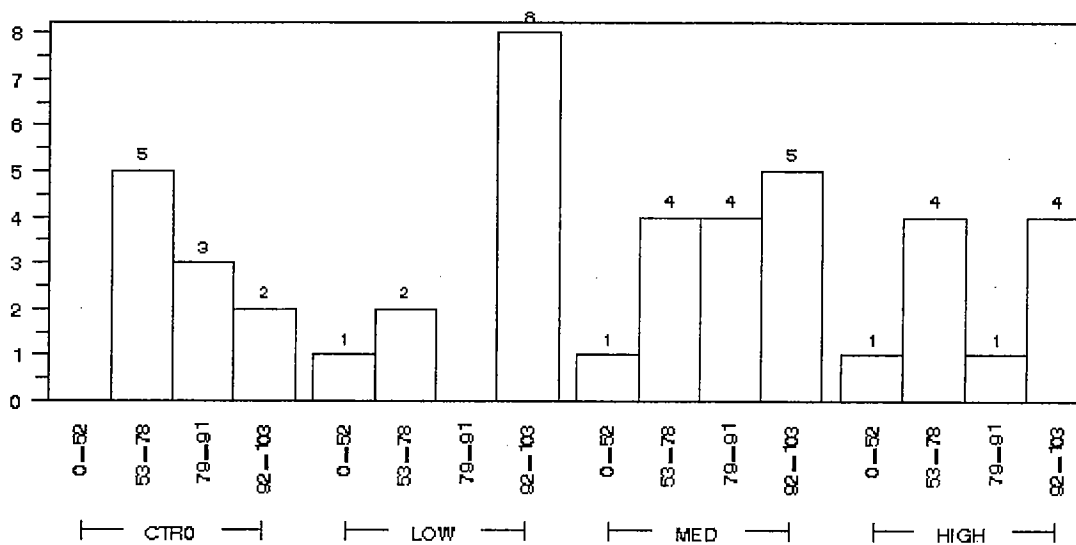
The mortality analysis starts with the display of the animal-mortality statistics by treatment and time interval. The main purpose for these analyses is to discover any statistically significant dose-mortality trend that justifies the age-adjusted test of positive dose-tumor linear trend.

Table 4 Analysis of Mortality Data for Male Rats by Treatment and Time

Analysis of Mortality		No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
CTR0	53-78	50	5	45	90.0	10.0
	79-91	45	3	42	84.0	16.0
	92-103	42	2	40	80.0	20.0
	FINALKILL104-105	40	40	0		
LOW	0-52	50	1	49	98.0	2.0
	53-78	49	2	47	94.0	6.0
	92-103	47	8	39	78.0	22.0
	FINALKILL104-105	39	39	0		
MED	0-52	50	1	49	98.0	2.0
	53-78	49	4	45	90.0	10.0
	79-91	45	4	41	82.0	18.0
	92-103	41	5	36	72.0	28.0
	FINALKILL104-105	36	36	0		
HIGH	0-52	50	1	49	98.0	2.0
	53-78	49	4	45	90.0	10.0
	79-91	45	1	44	88.0	12.0
	92-103	44	4	40	80.0	20.0
	FINALKILL104-105	40	40	0		

Source data: Analysis data (SAS v. 9.1) R2M21882

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Figure 1 Number of Male Rats Died During Study by Time

Source data: Analysis data (SAS v. 9.1) R2M21882

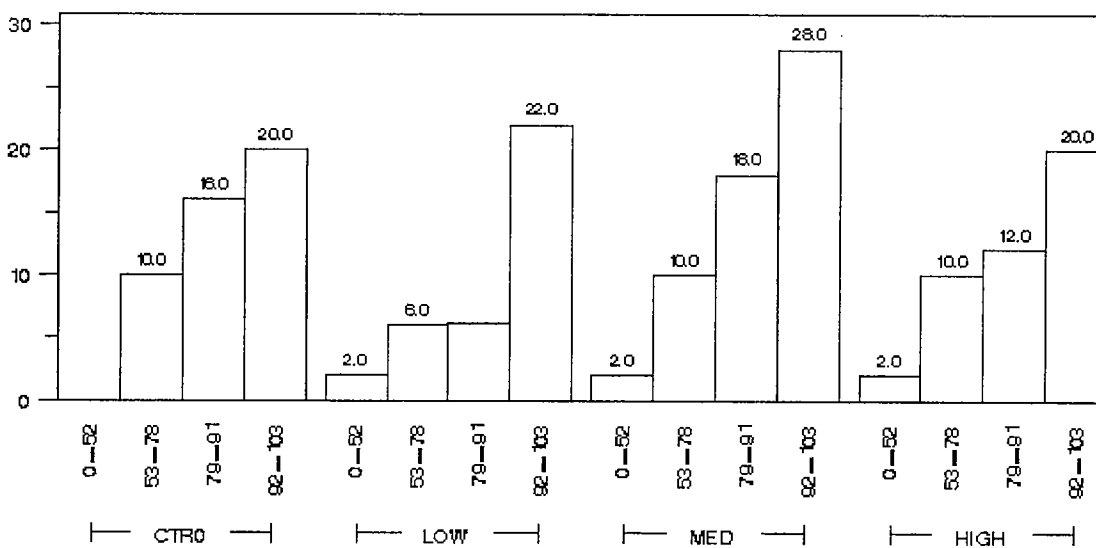
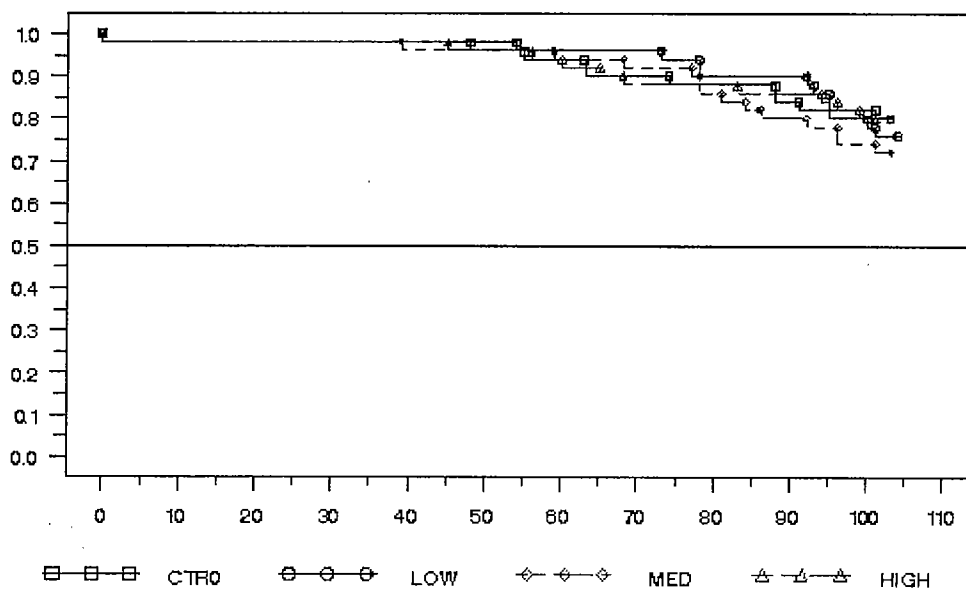
Figure 2 Cumulative Pct. of Death in Male Rats

Figure 3 Kaplan-Meier Survival Functions for Male Rats

Source data: Analysis data (SAS v. 9.1) R2M21882

The analysis of dose-mortality trend is done using a computer program described in the article "Trend and Homogeneity Analyses of Proportions and Life Table Data," Version 2.1, by Donald G. Thomas, National Cancer Institute. A significant dose-tumor trend gives rise to a statistical justification for the age-adjusted test of positive dose-tumor linear trend.

Table 5 Analysis of Dose-Mortality Trend for Male Rats

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test	1.1761	0.5554	1.1153	0.5726
Depart from Trend				
Dose-Mortality Trend	0.0048	0.9448	0.0109	0.9168
Homogeneity	1.1809	0.7576	1.1262	0.7707

Source data: Analysis data (SAS v. 9.1) R2M21882

Reviewer's Comment on Mortality Analysis:

The dose-mortality trend was found not to be statistically significant.

Trend Analysis

The test for positive dose-tumor linear trend is the ultimate objective of the evaluation of the carcinogenicity-study. We are looking for such trend because, in most situations, the carcinogenic potential of the test drug is unclear. Tumor incidences among the treatment groups appear random in many cases, while these incidences might be a result of the drug effect that needs to be detected and reported. Occasionally, pairwise comparisons are employed, but only under certain condition of the data and are decided on case by case bases. As a cautionary note, blindly imposing pairwise comparisons can only undermine the importance of the trend test, inflate the type-1 error, and produce untrustworthy results.

The significance of the test is decided based on a decision rule adopted by the Office of Biostatistics. The details of the decision rule can be found in the Appendix of this review.

We only report trend-test results with p-value less than 0.05, which may not imply a statistical significance.

Table 6 Report on Test for Positive Linear Dose-Tumor Trends in Male Rats: Combined Control

Organ Code	Organ Name	Tumor Code	Tumor Name	CTR0	LOW	MED	HIGH	P-Value (Exact Method)	P-Value (Asymptotic Method)
0002	ADRENAL	00043	CORTICAL ADENOMA [B].	0	0	1	2	0.0623	0.0341
0035	LUNG	00044	ADENOMA [B], bronchioloalveola	0	0	0	1	0.2105	0.0446

Source data: Analysis data (SAS v. 9.1) R2M21882

For exploration purposes, the following table gives an account of all tumor-bearing male rats by organ and tumor.

Table 7 Tumor-bearing male rats by organ and tumor

Organ code	Organ name	Tumor code	Tumor name	Ctrl	Doses in mg/kg/day		
					15	30	60
0002	ADRENAL	00014	PHEOCHROMOCYTOMA [B].	2	1	1	0
0002	ADRENAL	00043	CORTICAL ADENOMA [B].	0	0	1	2
0006	BONE	00034	OSTEOSARCOMA [M].	0	1	0	1
0010	BRAIN	00011	OLIGODENDROGLIOMA [M].	0	0	1	0
0010	BRAIN	00033	GRANULAR CELL TUMOR [M].	0	2	0	1
0010	BRAIN	00035	GRANULAR CELL TUMOR [B].	0	1	0	0
0010	BRAIN	00046	ASTROCYTOMA [M].	0	0	0	1
0015	COLON	00042	LEIOMYOSARCOMA [M].	0	0	1	0
0019	EPIDIDYMIDES	00036	MESOTHELIOMA [M].	0	2	2	0
0027	HEART	00026	NERVE SHEATH TUMOR [M], endocardium.	1	0	0	0
0028	HINDLIMB	00030	HEMANGIOSARCOMA [M].	0	1	0	0
0031	KIDNEY	00021	ADENOMA [B].	0	0	0	1
0031	KIDNEY	00040	TRANSITIONAL CELL CARCINOMA [M].	0	0	1	0

Organ code	Organ name	Tumor code	Tumor name	Ctrl	Doses in mg/kg/day		
					15	30	60
0031	KIDNEY	00041	LIPOSARCOMA [M].	0	0	1	0
0034	LIVER	00015	HEPATOCELLULAR ADENOMA [B].	0	1	1	0
0034	LIVER	00032	HEPATOCELLULAR CARCINOMA [M].	1	0	2	2
0035	LUNG	00044	ADENOMA [B], bronchioloalveolar.	0	0	0	1
0042	MEDIASTINAL LN	00030	HEMANGIOSARCOMA [M].	1	0	0	0
0043	MESENTERIC LN	00030	HEMANGIOSARCOMA [M].	0	0	2	0
0047	SUBLUMBAR LN	00030	HEMANGIOSARCOMA [M].	0	0	1	0
0049	MEDIASTINUM	00029	NERVE SHEATH TUMOR [M], cystic.	0	0	1	0
0051	MUSCLE	00030	HEMANGIOSARCOMA [M].	0	0	1	0
0051	MUSCLE	00038	FIBROSARCOMA [M].	0	0	1	0
0055	PANCREAS	00002	ISLET CELL CARCINOMA [M].	0	1	3	1
0055	PANCREAS	00018	ISLET CELL ADENOMA [B].	0	2	0	1
0056	PARATHYROID	00021	ADENOMA [B].	1	1	1	0
0057	PITUITARY	00001	ADENOMA [B], pars distalis.	15	19	15	20
0057	PITUITARY	00005	ADENOMA [B], pars intermedia.	1	2	0	0
0057	PITUITARY	00045	CARCINOMA [M], pars distalis.	0	0	0	1
0059	PROSTATE	00019	ADENOCARCINOMA [M].	1	0	1	0
0059	PROSTATE	00021	ADENOMA [B].	1	1	0	0
0060	RECTUM	00037	SARCOMA [M]	0	1	0	0
0061	SALIVARY GL	00019	ADENOCARCINOMA [M].	0	0	1	0
0063	SKIN	00004	FIBROMA [B].	2	0	0	1
0063	SKIN	00006	LIPOMA [B].	1	0	0	0
0063	SKIN	00009	PAPILLOMA [B].	1	1	0	0
0063	SKIN	00010	KERATOACANTHOMA [B].	5	2	2	1
0063	SKIN	00023	NERVE SHEATH TUMOR [M].	0	0	0	1
0063	SKIN	00029	NERVE SHEATH TUMOR [M], cystic.	1	0	0	0
0063	SKIN	00030	HEMANGIOSARCOMA [M].	0	0	1	0
0063	SKIN	00038	FIBROSARCOMA [M].	0	0	0	1
0063	SKIN	00039	BASAL CELL TUMOR [B].	0	0	2	1
0065	SPLEEN	00030	HEMANGIOSARCOMA [M].	0	0	1	0
0067	SYSTEMIC	00024	HISTIOCYTIC SARCOMA [M].	0	1	0	0
0067	SYSTEMIC	00027	LYMPHOMA [M].	1	1	0	0
0069	TESTIS	00007	INTERSTITIAL CELL TUMOR [B].	3	1	1	0
0071	THYMUS	00017	THYMOMA [B].	1	0	1	3
0071	THYMUS	00028	THYMOMA [M].	1	0	0	0
0072	THYROID	00008	FOLLICULAR ADENOMA [B].	5	4	2	0
0072	THYROID	00020	C-CELL ADENOMA [B].	9	7	8	9
0072	THYROID	00031	C-CELL CARCINOMA [M].	1	0	2	0
0081	ZYMBAL'S GL	00025	CARCINOMA [M].	2	0	0	0

Source data: Analysis data (SAS v. 9.1) R2M21882

Reviewer's Statistical Findings from the Trend-Test:

The dose-tumor linear trend in male rats was not statistically significant in any of the reported tumors.

Analysis of Female Rats

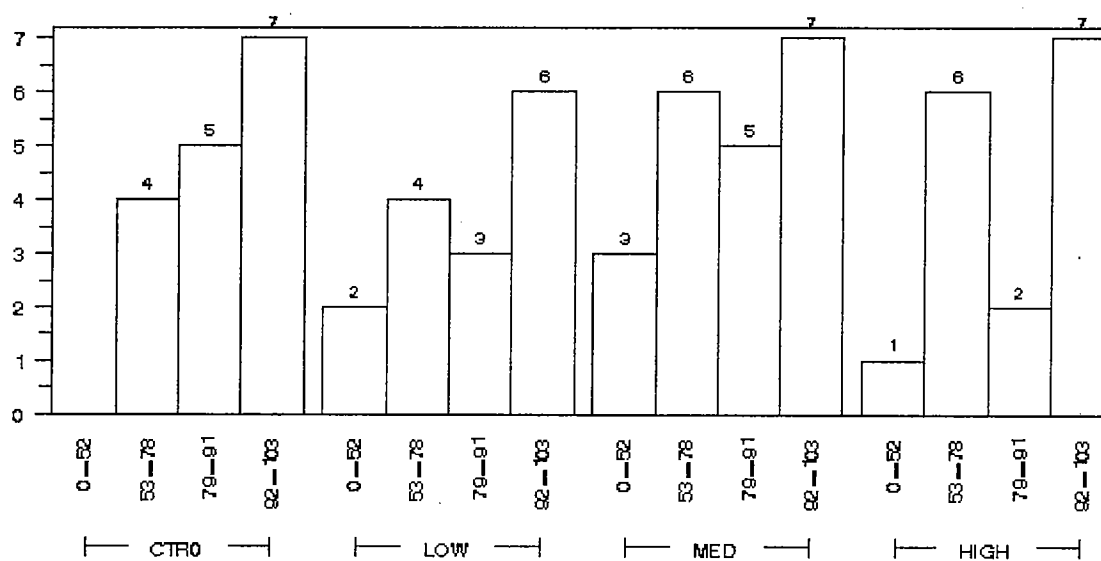
Mortality Analysis

The mortality analysis starts with the display of the animal-mortality statistics by treatment and time interval. The main purpose for these analyses is to discover any statistically significant dose-mortality trend that justifies the age-adjusted test of positive dose-tumor linear trend.

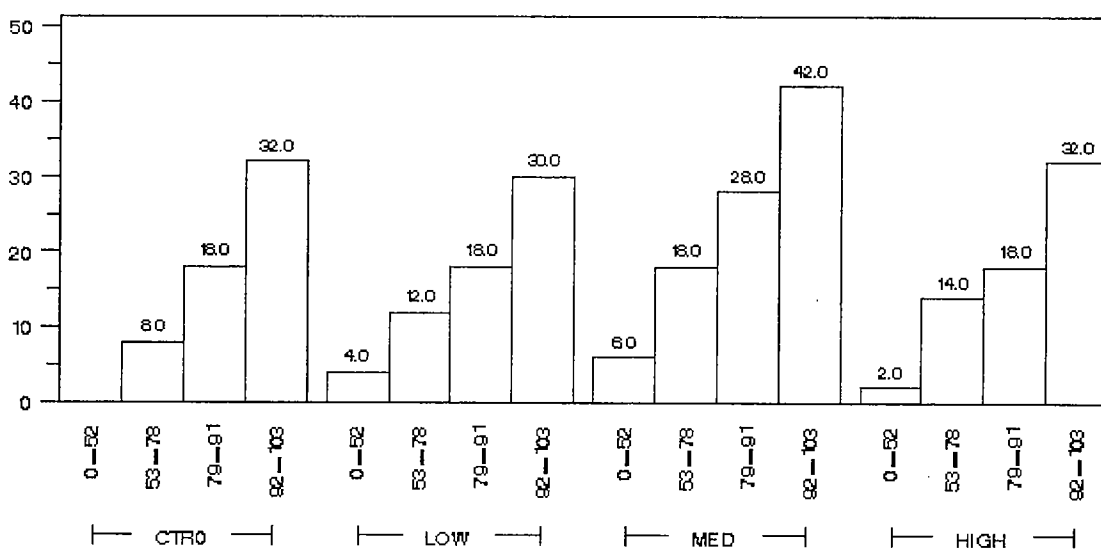
Table 8 Analysis of Mortality Data for Female Rats by Treatment and Time

Analysis of Mortality		No. Risk	No. Died	No. Alive	Pct Survival	Pct Mortality
CTR0	53-78	50	4	46	92.0	8.0
	79-91	46	5	41	82.0	18.0
	92-103	41	7	34	68.0	32.0
	FINALKILL104-105	34	34	0		
LOW	0-52	50	2	48	96.0	4.0
	53-78	48	4	44	88.0	12.0
	79-91	44	3	41	82.0	18.0
	92-103	41	6	35	70.0	30.0
	FINALKILL104-105	35	35	0		
MED	0-52	50	3	47	94.0	6.0
	53-78	47	6	41	82.0	18.0
	79-91	41	5	36	72.0	28.0
	92-103	36	7	29	58.0	42.0
	FINALKILL104-105	29	29	0		
HIGH	0-52	50	1	49	98.0	2.0
	53-78	49	6	43	86.0	14.0
	79-91	43	2	41	82.0	18.0
	92-103	41	7	34	68.0	32.0
	FINALKILL104-105	34	34	0		

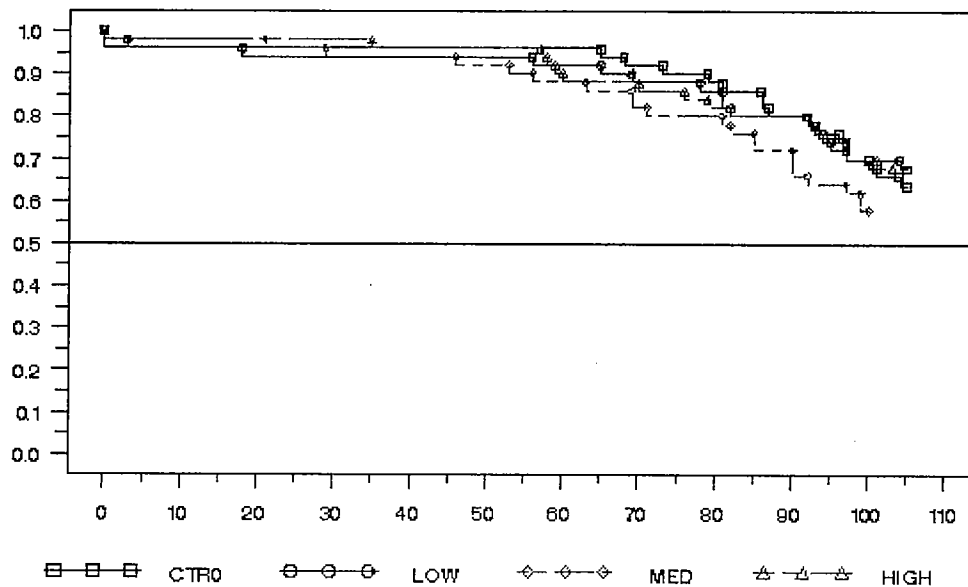
Source data: Analysis data (SAS v. 9.1) R2F21882

Figure 4 Number of Female Rats Died During Study by Time

Source data: Analysis data (SAS v. 9.1) R2F21882

Figure 5 Cumulative Pct. of Death in Female Rats

Source data: Analysis data (SAS v. 9.1) R2F21882

Figure 6 Kaplan-Meier Survival Functions for Female Rats

Source data: Analysis data (SAS v. 9.1) R2F21882

The analysis of dose-mortality trend is done using a computer program described in the article "Trend and Homogeneity Analyses of Proportions and Life Table Data," Version 2.1, by Donald G. Thomas, National Cancer Institute. A significant dose-tumor trend gives rise to a statistical justification for the age-adjusted test of positive dose-tumor linear trend.

Table 9 Analysis of Dose-Mortality Trend for Female Rats

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test	2.2409	0.3261	2.4399	0.2953
Depart from Trend				
Dose-Mortality Trend	0.0667	0.7962	0.1015	0.7501
Homogeneity	2.3076	0.5111	2.5413	0.4679

Source data: Analysis data (SAS v. 9.1) R2F21882

Reviewer's Comment on Mortality Analysis:

The dose-mortality trend was found not to be statistically significant.

Trend Analysis

The test for positive dose-tumor linear trend is the ultimate objective of the evaluation of the carcinogenicity-study. We are looking for such trend because, in most situations, the carcinogenic potential of the test drug is unclear. Tumor incidences among the treatment groups appear random in many cases, while these incidences might be a result of the drug effect that needs to be detected and reported. Occasionally, pairwise comparisons are employed, but only under certain condition of the data and are decided on case by case bases. As a cautionary note, blindly impose pairwise comparisons can only undermine the importance of the trend test, inflate the type-1 error, and produce untrustworthy results. The significance of the test is decided based on a decision rule adopted by the Office of Biostatistics. The details of the decision rule can be found in the Appendix of this review.

We only report trend-test results with p-value less than 0.05, which may not imply a statistical significance.

Table 10 Report on Test for Positive Linear Dose-Tumor Trends in Female Rats: Combined Control

No report was produced for all the trend-tests resulted in p-values greater or equal to 0.05.

For exploration purposes, the following table gives an account of all tumor-bearing female rats by organ and tumor.

Table 11 Tumor-bearing female rats by organ and tumor

Organ code	Organ name	Tumor code	Tumor name	Ctrl	Doses in mg/kg/day		
					15	30	60
0002	ADRENAL	00014	PHEOCHROMOCYTOMA [B].	0	1	0	0
0002	ADRENAL	00030	HEMANGIOSARCOMA [M].	1	0	0	0
0002	ADRENAL	00043	CORTICAL ADENOMA [B].	1	0	1	1
0002	ADRENAL	00053	PHEOCHROMOCYTOMA [M].	1	0	0	0
0010	BRAIN	00011	OLIGODENDROGLIOMA [M].	0	0	1	0
0010	BRAIN	00035	GRANULAR CELL TUMOR [B].	0	0	0	1
0010	BRAIN	00046	ASTROCYTOMA [M].	0	2	0	1
0012	CECUM	00042	LEIOMYOSARCOMA [M].	1	0	1	0
0013	CERVIX	00042	LEIOMYOSARCOMA [M].	1	0	0	0
0013	CERVIX	00054	POLYP [B].	1	0	0	0
0014	CLITORAL GL	00059	SQUAMOUS CELL CARCINOMA [M].	0	0	0	1
0027	HEART	00026	NERVE SHEATH TUMOR [M], endocardium.	0	1	0	2
0034	LIVER	00015	HEPATOCELLULAR ADENOMA [B].	2	3	0	0
0034	LIVER	00032	HEPATOCELLULAR CARCINOMA [M].	1	1	0	0
0034	LIVER	00056	CHOLANGIOMA [B].	0	1	0	0
0035	LUNG	00044	ADENOMA [B], bronchioloalveolar.	1	1	0	1
0043	MESENTERIC LN	00030	HEMANGIOSARCOMA [M].	1	1	0	0
0048	MAMMARY GL	00004	FIBROMA [B].	1	0	0	0

Organ code	Organ name	Tumor code	Tumor name	Ctrl	Doses In mg/kg/day		
					15	30	60
0048	MAMMARY GL	00019	ADENOCARCINOMA [M].	3	2	3	1
0048	MAMMARY GL	00021	ADENOMA [B].	1	0	0	1
0048	MAMMARY GL	00047	FIBROADENOMA [B].	12	8	3	8
0051	MUSCLE	00055	RHABDOMYOSARCOMA [M].	0	1	0	0
0054	OVARY	00019	ADENOCARCINOMA [M].	1	0	0	0
0054	OVARY	00051	THECAL CELL TUMOR [M].	1	0	0	0
0054	OVARY	00052	THECAL CELL TUMOR [B].	1	0	0	0
0054	OVARY	00058	LUTEAL CELL TUMOR [B].	0	0	0	1
0056	PARATHYROID	00021	ADENOMA [B].	1	2	0	0
0057	PITUITARY	00001	ADENOMA [B], pars distalis.	29	22	28	28
0057	PITUITARY	00005	ADENOMA [B], pars intermedia.	1	0	0	0
0057	PITUITARY	00045	CARCINOMA [M], pars distalis.	0	2	0	0
0063	SKIN	00003	BASAL CELL CARCINOMA [M].	1	0	0	0
0063	SKIN	00004	FIBROMA [B].	0	0	0	1
0063	SKIN	00006	LIPOMA [B].	0	1	0	0
0063	SKIN	00009	PAPILLOMA [B].	0	0	1	0
0063	SKIN	00010	KERATOACANTHOMA [B].	0	0	1	0
0063	SKIN	00038	FIBROSARCOMA [M].	0	1	0	1
0063	SKIN	00039	BASAL CELL TUMOR [B].	1	0	0	1
0063	SKIN	00060	SEBACEOUS CARCINOMA [M].	0	0	0	1
0066	STOMACH	00037	SARCOMA [M]	0	0	0	1
0066	STOMACH	00042	LEIOMYOSARCOMA [M].	0	0	0	1
0066	STOMACH	00059	SQUAMOUS CELL CARCINOMA [M].	0	0	0	1
0067	SYSTEMIC	00024	HISTIOCYTIC SARCOMA [M].	0	3	0	0
0067	SYSTEMIC	00027	LYMPHOMA [M].	0	0	1	0
0071	THYMUS	00017	THYMOMA [B].	4	5	5	6
0071	THYMUS	00028	THYMOMA [M].	1	0	1	1
0072	THYROID	00008	FOLLICULAR ADENOMA [B].	3	1	1	1
0072	THYROID	00020	C-CELL ADENOMA [B].	13	6	7	5
0072	THYROID	00031	C-CELL CARCINOMA [M].	0	1	0	0
0076	UTERUS	00019	ADENOCARCINOMA [M].	2	2	1	2
0076	UTERUS	00048	ENDOMETRIAL STROMAL SARCOMA [M].	1	2	0	0
0076	UTERUS	00049	ENDOMETRIAL STROMAL POLYP [B].	5	5	7	3
0076	UTERUS	00057	CHORIOCARCINOMA [M].	0	0	0	1
0079	VAGINA	00035	GRANULAR CELL TUMOR [B].	2	0	0	0
0079	VAGINA	00054	POLYP [B].	2	1	0	0
0081	ZYMBAL'S GL	00025	CARCINOMA [M].	0	0	0	1

Source data: Analysis data (SAS v. 9.1) R2F21882

Reviewer's Statistical Findings from the Trend-Test:

The dose-tumor linear trend in male rats was not statistically significant in any of the reported tumors.

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CC:

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Concur with review



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

Statistical Review and Evaluation

CARCINOGENICITY STUDY

BLA/Serial Number: NDA 21-882
Drug Name: Exajade (desferasirox)/Iron Chelator ICL670
Indication(s):
Applicant: Novartis
Date(s): Applicant's letter date: June 29, 2005
Review Priority: Standard
Biometrics Division: Biometrics Division 2
Statistical Reviewer: Ted Guo, Ph.D. (HFD-715)
Concurring Reviewers: Karl Lin, Ph.D., Biometrics Division 2
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Keywords: NDA review, Carcinogenicity

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EXECUTIVE SUMMARY

The analyses of carcinogenicity potential in male and female p53 heterozygous mice fed with iron supplement did not show a positive linear dose-tumor trend when the positive control group was excluded.

Please note that the numbers of organs and tissues that were marked as “not examined” in both sex groups of p53 heterozygous mice. Therefore, the dose-tumor-trend test was done based on a small number of organs. The conclusion of the negative findings of carcinogenic potential should be interpreted with caution.

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OVERVIEW OF CARCINOGENICITY STUDIES

The purpose of the submitted carcinogenicity study was “to evaluate the carcinogenic potential of ICL670, an iron chelator, when administered orally to p53 heterozygous mice and p53 wild type mice for 26 weeks (p. 10, 0270117.pdf).”

This reviewer does not understand how the drug, Exajade (desferasirox), and Iron Chelator ICL670, the chemical substance under investigation in Study 0270117 are related.

ICL670 (Batch no. 9923008) was administered orally via gavage as a suspension. It was either fed supplemented with iron or fed in standard diet. Two types of mice were included in this study: p53 heterozygous mice and p53 wild-type mice.

The dosage assignments for the mice are shown in the following two tables.

Table 1 ICL670 Dosage assignments supplemented with and without iron supplement

Species	Sex	No. Animals per group	Dosage (mg/kg/day)
P53 heterozygous mice	Male	25	0, 0(s), 20, 30(s), 100, 200, 400(p)
	Female	25	0, 0(s), 30(s), 30, 100→60(s), 100, 300, 400(p)
P53 wild-type mice	Male	25	0, 0(s), 200
	Female	25	0, 0(s), 300

Note that the dosage symbolized by 0(s) represents the feed in standard diet, i.e., with no ICL670 in the feed. “Due to excessive mortality in the unsupplemented females at 100→60 mg/kg/day, two additional female dose groups (25/group) were added to the study (0 mg/kg/day and 30 mg/kg/day, standard feed). An additional group of p53 heterozygous mice (25/sex) received p-cresidine, and served as a positive control (p. 10, 0270117.pdf).” The positive control is represented here by 400(p).

As conclusions, the sponsor indicated in the Summary, “Based on the results from this study, **ICL670 administered orally via gavage for 26 weeks was not carcinogenic in p53 heterozygous or wild type mice at doses up to 200 mg/kg/day for males and 300 mg/kg/day for females.** As expected, the administration of p-cresidine caused proliferative changes notably in the urinary bladder of p53 heterozygous mice (p. 13, 0270117.pdf).”

REVIEWER'S EVALUATION OF CARCINOGENICITY STUDIES

The statistical review is based on the sponsor's data submitted on June 29, 2005 upon request from the Agency on June 21, 2005. Three sets of data were submitted to the Agency.

Table 2 Definition files for the tumor data sets

 NOVARTIS	
NDA No. 21-882	
Exajade (desferasirox)	
125, 250, 500 mg dispersable tablets	
Indication: Treatment of chronic transfusional overload	
Case Report Tabulations	
Datasets Table of Contents	
Datasets	Location of File
Study No. 0270117	Crt\datsetsi0270117\0270117define.pdf
Study No. 0270117F	Crt\datsetsi0270117F\0270117Fdefine.pdf
Study No. 0270117W	Crt\datsetsi0270117W\0270117Wdefine.pdf

[-]  n21882 on OIT-DIMS NTSYS E-Submission (Park) (Cdsesub 1)
[-]  N_000
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[-]  0270117
[+]  Raw
[-]  0270117F
[+]  Raw
[-]  0270117W
[+]  Raw

Source: Datatoc.pdf

Because the mice in the same study were fed with different diet (with or without iron), and some mice were treated with a known carcinogen as the positive control, this reviewer took the following considerations in statistical analyses.

1. The positive control was excluded in the dose-tumor trend test.
2. In a single trend analysis, only those fed with iron were included.
3. Heterozygous and wild-type mice were analyzed separately. And for the wild-type mice, only pairwise comparisons were done.
4. This reviewer combined data in Study No. 0270117 and Study No. 0270117F in the same trend analysis. The information in Study No. 0270117F included only additional data to the two unsupplemented (fed with standard diet) treatment groups to compensate the excessive mortality in those groups.

The sponsor's data have been regulated as a common practice in the statistical review. Not an original data value has been altered or changed. The analysis data sets are listed in the following table.

Table 3 Analysis data files

Name	Explanation
M1M21882	Male-mouse data
M2F21882	Female-mouse data
M1M218_W	Male-wild-type-mouse data
M1F218_W	Female-wild-type-mouse data

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Analysis of Male p53 Heterozygous Mice

Evaluation of Organ/Tissue-examination status

It is expected that the sponsor submits a tumor-finding report for all organs and tissues. Leaving organs and tissues unexamined could result in untrustworthy conclusion about the carcinogenic potential. Leaving the majority of the organs and tissues unexamined could even invalidate the carcinogenicity study itself. The purpose for the organ usability analysis is to report the status of organ and tissue examinations.

Table 4 Number of Animals: Organs and Tissues Not Examined in Male P53 Heterozygous Mice by Organ/Tissue and Treatment

Organ/Tissue	0: Control	20 mg/kg/day	100 mg/kg/day	200 mg/kg/day	Total
ADRENAL	1				1
AORTA				1	1
ARTERY	24	25	25	25	99
AXILLARY LN	25	23	25	25	98
BRONCHIAL LN	4	2	2	4	12
DIAPHRAGM	25	25	24	25	99
EAR	25	25	25	25	100
FAT	25	25	25	25	100
FAT - MEDIAST	25	25	23	25	98
GALL BLADDER			1		1
HEAD	25	25	25	25	100
LARYNX	25	25	25	25	100
MAMMARY GLAND	23	24	24	25	96
MANDIBULAR LN	2	3		3	8
MEDIASTINAL LN	25	25	25	25	100
MEDIASTINUM	25	25	25	25	100
MESENTERIC LN		1	1	1	3
MESENTERY	25	25	25	25	100
NERVE - SCIATI	1	1		1	3
PANCREATIC LN	25	25	25	25	100
PARATHYROID	3	4	3	5	15
PREPUTIAL GL	6	3	2	2	13
RENAL LN	25	23	25	24	97
SUBLUMBAR LN	25	23	25	25	98
TAIL	25	25	25	25	100
THORACIC CAV	25	25	25	25	100
THYMUS	2				2
THYROID			1		1
TRACHEA			1		1

Source data: Analysis data (SAS v. 9.1) M1M21882; Program: Analysis2.sas

Table 5 Number of Animals: Organs/Tissues Examined in Male P53 Heterozygous Mice by Organ and Treatment

Organ/Tissue	20 mg/kg/day	100 mg/kg/day	Total
ADRENAL		1	1
SYSTEMIC	2		2

Source data: Analysis data (SAS v. 9.1) M1M21882; Program: Analysis2.sas

This table shows that **ADRENAL** and **SYSTEMIC** were examined and tumors were detected. Note that if an organ was examined and no tumors were found in that organ, that organ was not reported in the data as were shown in Tables 4 and 5, above.

Trend Analysis

The test for positive dose-tumor linear trend is the ultimate objective of the evaluation of the carcinogenicity-study. We are looking for such trend because, in most situations, the carcinogenic potential of the test drug is unclear. Tumor incidences among the treatment groups appear random in many cases, while these incidences might be a result of the drug effect that needs to be detected and reported. Occasionally, pairwise comparisons are employed, but only under certain condition of the data and are decided on case by case bases. As a cautionary note, blindly impose pairwise comparisons can only undermine the importance of the trend test, inflate the type-1 error, and produce untrustworthy results. The significance of the test is decided based on a decision rule adopted by the Office of Biostatistics.

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Table 6 Tumors Found in Male P53 Heterozygous Mice: Iron diet, positive Control Excluded

Organ code	Organ name	Tumor code	Tumor name	0: Control	20 mg/kg/day	100 mg/kg/day	200 mg/kg/day
0001	ADRENAL	00001	CORTICAL ADENOMA [B], bilateral.	0	0	0	0
0001	ADRENAL	00003	CORTICAL ADENOMA [B].	0	0	1	0
0001	ADRENAL	00008	PHEOCHROMOCYTOMA [B].	0	0	0	0
0015	EPIDIDYIMIDES	00004	LEIOMYOSARCOMA [M].	0	0	0	0
0021	HARDERIAN GL	00011	ADENOMA [B].	0	0	0	0
0029	LIVER	00009	TRANSITIONAL CELL CARCINOMA [M], meta	0	0	0	0
0053	SKIN	00007	CARCINOMA [M], not otherwise specific	0	0	0	0
0057	SYSTEMIC	00002	LYMPHOMA [M].	0	2	0	0
0066	URINARY BL	00005	TRANSITIONAL CELL CARCINOMA [M].	0	0	0	0
0066	URINARY BL	00006	TRANSITIONAL CELL PAPILLOMA [B].	0	0	0	0
0066	URINARY BL	00010	SQUAMOUS CELL CARCINOMA [M].	0	0	0	0

Source data: Analysis data (SAS v. 9.1) M1M21882

Table 7 Test for Positive Linear Dose-Tumor Trends in Male P53 Heterozygous Mice: Iron diet, positive Control Excluded

Tumor Name	Organ Name	Dosage	#Animals	P-value
LYMPHOMA [M]	SYSTEMIC	20 mg/kg/day	2	0.2475
CORTICAL ADENOMA [B]	ADRENAL	100 mg/kg/day	1	0.7500

Source data: Analysis data (SAS v. 9.1) M1M21882

Reviewer's Statistical Findings from the Trend-Test:

Statistically significant linear trend of dose-tumor in male P53 heterozygous mice was not detected in any of the reported tumors.

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Analysis of Female p53 Heterozygous Mice

Evaluation of Organ/Tissue-examination status

It is expected that the sponsor submits a tumor-finding report for all organs and tissues. Leaving organs and tissues unexamined could result in untrustworthy conclusion about the carcinogenic potential. Leaving the majority of the organs and tissues unexamined could even invalidate the carcinogenicity study itself. The purpose for the organ usability analysis is to report the status of organ and tissue examinations.

Table 8 Number of Animals: Organs and Tissues Not Examined in Female P53 Heterozygous Mice by Organ/Tissue and Treatment

Organ/Tissue	0: Control	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day	Total
AORTA		2		1	3
ARTERY	24	49	24	25	122
AXILLARY LN	25	25	25	25	100
BRONCHIAL		4			4
BRONCHIAL LN	3		2	6	11
CECUM	1				1
CLITORAL GL	15	23	14	15	67
DIAPHRAGM	25	25	25	25	100
DUODENUM				1	1
EAR	25	25	25	25	100
ESOPHAGUS				1	1
FAT	25	24	25	25	99
FAT - MEDIAST	25	25	25	22	97
GALL BLADDER		3	1	1	5
HEAD	25	25	25	24	99
ILEUM				1	1
JEJUNUM	1				1
KIDNEY		1			1
LACRIMAL GL		5		1	6
LARYNX	25	25	25	25	100
LIVER		1			1
LUNG				1	1
MAMMARY GLAND	1				1
MANDIBULAR		1			1
MANDIBULAR LN	1	2		3	6
MEDIASTINAL LN	24	25	25	25	99
MEDIASTINUM	25	49	25	25	124
MESENTERY	25	25	25	24	99
NERVE - SCIATI	1	1	1		3

Organ/Tissue	0: Control	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day	Total
NERVE SCIATIC		2			2
PANCREATIC LN	25	25	25	25	100
PARATHYROID	4	8	3	3	18
RENAL LN	25	25	25	25	100
SPLEEN		1			1
SUBLUMBAR LN	25	25	25	25	100
TAIL	25	25	25	25	100
THORACIC CAV	25	25	25	25	100
THYMUS			1		1
THYROID				1	1
TRACHEA				1	1
VERTEBRAE		25			25

Source data: Analysis data (SAS v. 9.1) M2F21882; Program: Analysis2.sas

Note that in the table, above, the numbers of female mice are based on those fed with both standard diet and iron supplement. This explains why the number of organs and tissues exceeds 25.

Table 9 Number of Animals: Organs/Tissues Examined in Female P53 Heterozygous Mice by Organ and Treatment

Organ/Tissue	0	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day	Total
MAMMARY GLAND			1		1
SKIN	1				1
SYSTEMIC	1	1			2
UTERUS				1	1

Source data: Analysis data (SAS v. 9.1) M2F21882; Program: Analysis2.sas

This table shows that **MAMMARY GLAND**, **SKIN**, **SYSTEMIC**, and **UTERUS** were examined and tumors were detected. Note that if an organ was examined and no tumors were found in that organ, that organ was not reported in the data as were shown in Tables 8 and 9, above.

Trend Analysis

The test for positive dose-tumor linear trend is the ultimate objective of the evaluation of the carcinogenicity-study. We are looking for such trend because, in most situations, the carcinogenic potential of the test drug is unclear. Tumor incidences among the treatment groups appear random in many cases, while these incidences might be a result of the drug effect that needs to be detected and reported. Occasionally, pairwise comparisons are employed, but only under certain condition of the data and are decided on case by case bases. As a cautionary note, blindly impose pairwise comparisons can only undermine the importance of the trend test, inflate the type-1 error, and produce untrustworthy results. The significance of the test is decided based on a decision rule adopted by the Office of Biostatistics.

Table 10 Tumors Found in Female P53 Heterozygous Mice: Iron diet, positive Control Excluded

Organ code	Organ name	Tumor code	Tumor name	0: Control	30 mg/kg/day	100 mg/kg/day	300 mg/kg/day
0004	FEMUR/TIBIA	00012	OSTEOSARCOMA [M].	0	0	0	0
0029	LIVER	00016	HEPATOCELLULAR ADENOMA [B].	0	0	0	0
0037	SALIVARY GL	00001	LYMPHOMA [M].	0	0	0	0
0039	MAMMARY GLAND	00013	ADENOCARCINOMA [M].	0	0	1	0
0042	SYSTEMIC	00001	LYMPHOMA [M].	0	0	0	0
0045	PANCREAS	00014	ACINAR CELL ADENOCARCINOMA [M].	0	0	0	0
0053	SKIN	00012	OSTEOSARCOMA [M].	1	0	0	0
0057	SYSTEMIC	00002	LYMPHOMA [M].	1	1	0	0
0065	UTERUS	00012	OSTEOSARCOMA [M].	0	0	0	1
0066	URINARY BL	00005	TRANSITIONAL CELL CARCINOMA [M].	0	0	0	0
0066	URINARY BL	00006	TRANSITIONAL CELL PAPILLOMA [B].	0	0	0	0
0066	URINARY BL	00015	PAPILLOMA [B], squamous cell.	0	0	0	0

Source data: Analysis data (SAS v. 9.1) M2F21882

Table 11 Test for Positive Linear Dose-Tumor Trends in Female P53 Heterozygous Mice: Iron diet, positive Control Excluded

Organ Name	Tumor Name	Dosage	# Animals	P-value
MAMMARY GLAND	ADENOCARCINOMA [M]	100 mg/kg/day	1	0.8000
SKIN	OSTEOSARCOMA [M]	Control	1	0.2000
SYSTEMIC	LYMPHOMA [M]	Control	1	0.2000
		30 mg/kg/day	1	0.6000
UTERUS	OSTEOSARCOMA [M]	300 mg/kg/day	1	1.0000

Source data: Analysis data (SAS v. 9.1) M2F21882

Reviewer's Statistical Findings from the Trend-Test:

Statistically significant linear trend of dose-tumor in female P53 heterozygous mice was not detected in any of the reported tumors.

Analysis of Male Wild-Type Mice

Evaluation of Organ/Tissue-examination status

It is expected that the sponsor submits a tumor-finding report for all organs and tissues. Leaving organs and tissues unexamined could result in untrustworthy conclusion about the carcinogenic potential. Leaving the majority of the organs and tissues unexamined could even invalidate the carcinogenicity study itself. The purpose for the organ usability analysis is to report the status of organ and tissue examinations.

Table 12 Number of Animals: Organs and Tissues Not Examined in Male Wild-Type Mice by Organ/Tissue and Treatment

Organ/Tissue	0: Control	200 mg/kg/day	Total
AORTA		1	1
ARTERY	25	25	50
AXILLARY LN	25	25	50
BRONCHIAL LN	2		2
DIAPHRAGM	25	25	50
EAR	25	25	50
FAT	25	25	50
FAT MEDIAST.	25	25	50
GALL BLADDER		1	1
HEAD	25	25	50
LARYNX	25	25	50
MAMMARY GLAND	22	24	46
MANDIBULAR LN	2	3	5
MEDIASTINAL LN	25	25	50
MESENTERY	25	25	50
PANCREATIC LN	25	25	50
PARATHYROID	3	2	5
PROSTATE	1		1
RENAL LN	25	25	50
SPLEEN	1		1
SUBLUMBAR LN	25	25	50
TAIL	25	25	50
THORACIC CAV	25	25	50
THYMUS	1		1

Source data: Analysis data (SAS v. 9.1) M1M218_W; Program: Analysis2.sas

Table 13 Number of Animals: Organs/Tissues Examined in Male Wild-Type Mice by Organ and Treatment

Organ/Tissue	Control	200 mg/kg/day	Total
SYSTEMIC		1	1

Source data: Analysis data (SAS v. 9.1) M1M218_W; Program: Analysis2.sas

This table shows that **SYSTEMIC** was examined and tumors were detected. Note that if an organ was examined and no tumors were found in that organ, that organ was not reported in the data as were shown in Tables 12 and 13, above.

Trend Analysis

The test for positive dose-tumor linear trend is the ultimate objective of the evaluation of the carcinogenicity-study. We are looking for such trend because, in most situations, the carcinogenic potential of the test drug is unclear. Tumor incidences among the treatment groups appear random in many cases, while these incidences might be a result of the drug effect that needs to be detected and reported. Occasionally, pairwise comparisons are employed, but only under certain condition of the data and are decided on case by case bases. As a cautionary note, blindly impose pairwise comparisons can only undermine the importance of the trend test, inflate the type-1 error, and produce untrustworthy results. The significance of the test is decided based on a decision rule adopted by the Office of Biostatistics.

Table 14 Tumors Found in Male Wild-Type Mice: Iron diet

Organ code	Organ name	Tumor code	Tumor name	0: Control	200 mg/kg/day
0056	SYSTEMIC	00001	MAST CELL TUMOR [B]	0	1

Source data: Analysis data (SAS v. 9.1) M1M218_W

Table 15 Test for Positive Linear Dose-Tumor Trends in Male Wild-Type Mice: Iron diet

Organ Name	Tumor Name	Dosage	#Animals	P-value
SYSTEMIC	MAST CELL TUMOR [B]	200 mg/kg/day	1	1.0000

Source data: Analysis data (SAS v. 9.1) M1M218_W

Reviewer's Statistical Findings from the Trend-Test:

Statistically significant linear trend of dose-tumor in male wild-type mice was not detected in any of the reported tumors.

Analysis of Female Wild-Type Mice

Evaluation of Organ/Tissue-examination status

It is expected that the sponsor submits a tumor-finding report for all organs and tissues. Leaving organs and tissues unexamined could result in untrustworthy conclusion about the carcinogenic potential. Leaving the majority of the organs and tissues unexamined could even invalidate the carcinogenicity study itself. The purpose for the organ usability analysis is to report the status of organ and tissue examinations.

Table 16 Number of Animals: Organs and Tissues Not Examined in Female Wild-Type Mice by Organ/Tissue and Treatment

Organ/Tissue	Control	300 mg/kg/day	Total
ARTERY	25	24	49
AXILLARY LN	24	25	49
CLITORAL GL	5	4	9
DIAPHRAGM	25	25	50
EAR	25	25	50
FAT	25	25	50
FAT MEDIAST.	25	25	50
HEAD	25	25	50
LACRIMAL GLAND	1	1	2
LARYNX	25	24	49
MEDIASTINAL LN	24	25	49
MESENTERIC LN		1	1
MESENTERY	25	25	50
PANCREATIC LN	24	25	49
PARATHYROID	2		2
PITUITARY		1	1
RENAL LN	24	25	49
SUBLUMBAR LN	24	25	49
TAIL	25	25	50
THORACIC CAV	25	25	50

Source data: Analysis data (SAS v. 9.1) M1F218_W; Program: Analysis2.sas

Table 17 Number of Animals: Organs/Tissues Examined in Female Wild-Type Mice by Organ and Treatment

Organ/Tissue	Control	Total
BRONCHIAL LN	1	1
SYSTEMIC	2	2
UTERUS	1	1

Source data: Analysis data (SAS v. 9.1) M1F218_W; Program: Analysis2.sas

This table shows that **BRONCHIAL LN** and **SYSTEMIC** were examined and tumors were detected. Note that if an organ was examined and no tumors were found in that organ, that organ was not reported in the data as were shown in Tables 16 and 17, above.

Trend Analysis

The test for positive dose-tumor linear trend is the ultimate objective of the evaluation of the carcinogenicity-study. We are looking for such trend because, in most situations, the carcinogenic potential of the test drug is unclear. Tumor incidences among the treatment groups appear random in many cases, while these incidences might be a result of the drug effect that needs to be detected and reported. Occasionally, pairwise comparisons are employed, but only under certain condition of the data and are decided on case by case bases. As a cautionary note, blindly impose pairwise comparisons can only undermine the importance of the trend test, inflate the type-1 error, and produce untrustworthy results. The significance of the test is decided based on a decision rule adopted by the Office of Biostatistics.

Table 18 Tumors Found in Female Wild-Type Mice: Iron diet

Organ code	Organ name	Tumor code	Tumor name	Control	300 mg/kg/day
0032	BRONCHIAL LN	00003	LYMPHOMA [M].	1	0
0056	SYSTEMIC	00003	LYMPHOMA [M].	1	0
0056	SYSTEMIC	00004	HISTIOCYTIC SARCOMA [M].	1	0
0064	UTERUS	00002	ENDOMETRIAL STROMAL POLYP [B].	1	0

Source data: Analysis data (SAS v. 9.1) M1F218_W

Table 19 Test for Positive Linear Dose-Tumor Trends in Female Wild-Type Mice: Iron diet

There is no need for the trend test.

Reviewer's Statistical Findings from the Trend-Test:

Statistically significant linear trend of dose-tumor in female wild-type mice was not detected in any of the reported tumors

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