

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

203568Orig1s000

STATISTICAL REVIEW(S)



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

STATISTICAL REVIEW AND EVALUATION

CLINICAL STUDIES

NDA: 203568

Drug Name: Kynamo (Mipomersen sodium injection)

Indications: 1. Adjunctive therapy to maximally tolerated lipid lowering medications and diet to reduce low density lipoprotein-cholesterol, apolipoprotein B , total cholesterol , non high-density lipoprotein cholesterol , and lipoprotein.
2. Treat homozygous familial hypercholesterolemia.

Applicants: Sponsor: Genzyme Corporation
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CRO: [REDACTED] (b) (4)

Date(s): Submitted: 29 March 2012
To Reviewer: 9 May 2012

Review Priority: Standard

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Keywords: Carcinogenicity, Cox regression, Kaplan-Meier product limit, Survival analysis, Trend test

Table of Contents

1. EXECUTIVE SUMMARY	3
1.1. CONCLUSIONS AND RECOMMENDATIONS	3
1.2. BRIEF OVERVIEW OF THE STUDIES	11
1.3. STATISTICAL ISSUES AND FINDINGS	11
1.3.1. <i>Statistical Issues</i>	11
1.3.2. <i>Statistical Findings</i>	16
2. INTRODUCTION	16
2.1. OVERVIEW	16
2.2. DATA SOURCES	16
3. STATISTICAL EVALUATION	17
3.1. EVALUATION OF EFFICACY	17
3.2. EVALUATION OF SAFETY	17
4. FINDINGS IN SPECIAL/SUBGROUP POPULATIONS	35
5. SUMMARY AND CONCLUSIONS	35
5.1. STATISTICAL ISSUES AND COLLECTIVE EVIDENCE	35
5.2. CONCLUSIONS AND RECOMMENDATIONS	35
APPENDICES.....	36
APPENDIX 1. FDA SURVIVAL ANALYSIS	36
APPENDIX 2. FDA POLY-K TUMORIGENICITY ANALYSIS	41
APPENDIX 3. REFERENCES.....	69

1. EXECUTIVE SUMMARY

Reports from two studies, in rats and mice, were provided. Both studies were conducted (b) (4). The rat report states that this “study was conducted to evaluate the potential carcinogenicity of the test article, ISIS 301012 (human-specific apoB inhibitor), in Sprague-Dawley rats after once weekly treatment administered via subcutaneous injection for up to 2 years. Three treatment groups of 60 male and 60 female CD® [CrI:CD®(SD)] rats were administered ISIS 301012 at dose levels of 3, 10, and 30/25/20 (males) or 25/20 (females) mg/kg/week and one group of 60 animals/sex received the rat-specific analog, ISIS 147768 at a dose level of 10 mg/kg/week.” (page 11 of rat report) The mouse report states that: “The objective of this study was to evaluate the potential carcinogenicity of ISIS 301012 (human-specific apoB inhibitor) and characterize the potential carcinogenic effects of a mouse specific apoB inhibitor (ISIS 147764) in CD-1 mice after once weekly treatment administered via subcutaneous injection for 2 years. Both ISIS 301012 and ISIS 147764 were evaluated to characterize the potential effects of the compound intended for clinical use (ISIS 301012).” (page 16 of mouse report)

1.1. Conclusions and Recommendations

The Sponsor describes the drug vehicle as phosphate buffered saline (PBS). The test articles were labeled as ISIS 301012 and ISIS 147768, were provided by ISIS Pharmaceuticals. “The vehicle and test articles were administered as received, and no dose preparation was performed (b) (4).” (page 19 of rat report) Gross aspects of the study designs for the main study animals are summarized below:

Table 1. Design of Albino Rat Study (60 animals per main study group/gender)

Treatment Group	Vehicle or Test Article	Male Dosage (mg/kg)	Dose Volume (mL/kg)	Dosing Interval (weeks) ^a	Female Dosage (mg/kg)	Dose Volume (mL/kg)	Dosing Interval (weeks) ^a
1. Vehicle	PBS	0	5/4.17/2 ^b	1 to 105	0	5/4.17/2 ^b	1 to 105
2. Low	ISIS 301012	3	2	1 to 105	3	2 ^f	1 to 96
3. Medium	ISIS 301012	10	2	1 to 94 ^e	10	2 ^f	1 to 98
4. High	ISIS 301012	30/25/20 ^c	5/4.17/2/1.6 ^d	1 to 70 ^e	25/20 ^a	4.17/2/1.6 ^{df}	1 to 88
5. 147768 ^b	ISIS 147768	10	2	8 to 105	10	2	8 to 105

^a Dosing ceased when survival reached 20 within a sex in a particular group, with terminal necropsy then occurring when survival subsequently reached 15.

^b The dose volume for control animals was reduced to 4.17 mL/kg based on the dose level reduction of Group 4 males during Week 2 and to 2 mL/kg based on the Group 4 test article concentration increase during Week 13.

^c The dose level for male animals was reduced to 25 mg/kg/week during Week 2 and for all animals to 20 mg/kg/week during Week 25.

^d The dose volume for males was reduced to 4.17 mL/kg based on the dose level reduction during Week 2 and for all animals to 2 mL/kg based on the dose concentration increase of 6 mg/mL to 12.5 mg/mL during Week 13 and to 1.6 mL/kg based on the dose level reduction during Week 25.

^e Males: Group 3 were necropsied at Week 96, Group 4 at week 74.

^f Females: Group 2 were necropsied at Week 98, Group 3 at week 100, Group 4 at week 92.

Table 2. Design of Mouse Study (70 animals per main study group/gender)

Treatment Group	Vehicle or Test Article	Dosage (mg/kg)	Frequency of Dosing	Male Dosing Interval (weeks) ^a	Female Dosing Interval (weeks) ^a
1. Vehicle	PBS	0	Once Weekly	1 to 105	1 to 105
2. Low	ISIS 301012	5	Once Weekly	1 to 105	1 to 105
3. Medium	ISIS 301012	20	Once Weekly	1 to 105	1 to 98 ^b
4. High	ISIS 301012	60	Once Weekly	1 to 95 ^b	1 to 83 ^b
5. Monthly	ISIS 301012	80	Once Monthly	1 to 105	1 to 105
6. 147764	ISIS 147764	60	Once Weekly	1 to 105	1 to 105

^a Dosing ceased when survival reached 20 within a sex in a particular group, with terminal necropsy then occurring when survival subsequently reached 15.

^b Group 4 males were sacrificed at Week 95, Group 3 females at week 98, Group 4 females at week 83.

Note that “[b]y examining the effects of the sequence intended for clinical use (ISIS 301012) as well as a murine active sequence, information was obtained on both the potential effects of the chemical class and those attributable to the pharmacologic activity.” (page 16 of mouse report)

More detailed descriptions of the studies are provided in Section 3.2.1 and 3.2.2 below. In this report the vehicle group is sometimes referred to as the “PBS” or “control group” while the other dose groups are referred to as “actual dose groups”, and, purposes of assessing trend, the Vehicle, Low, Medium, and High dose groups (i.e. Groups 1-4) as “treated groups.” Simple summary life tables in mortality are presented in the report in these sections of this report.

In Appendix 1, Figures A.1.1 and A.1.2 for rats and Figures A.1.3 and A.1.4 for mice, display Kaplan-Meier estimated survival curves for each study group for each species and gender combination. The results of the tests of trend and differences in survival are displayed in Tables 3 and 4 below:

Table 3. Statistical Significances of Tests of Homogeneity and Trend in Survival in the Rat Study

Hypothesis Tested	Males		Females	
	Log rank	Wilcoxon	Log rank	Wilcoxon
Rat Homogeneity over Groups 1-5	< 0.0001	< 0.0001	0.0022	0.0114
Homogeneity over Groups 1-4	< 0.0001	< 0.0001	0.0006	0.0060
No trend over Groups 1-4	< 0.0001	< 0.0001	0.0003	0.0023
Homogeneity over Groups 1-3	0.0001	0.0005	0.0489	0.2197
No trend over Groups 1-3	< 0.0001	0.0002	0.1702	0.4727
No Difference Between Groups 1 vs 3	< 0.0001	< 0.0001	0.0802	0.2924
No Difference Between Groups 1 vs 4	< 0.0001	< 0.0001	0.0023	0.0046
No Difference Between Groups 1 vs 5	0.6482	0.6580	0.2447	0.3097

From Figure A.1.1, in male rats, at first there is a clear decreasing survival over dose with the exception that Group 5, the ISIS 147768 group, closely tracks the group with the highest survival, the vehicle group, Group 1. This is consistent with the results of the tests above. With the exception of the pairwise comparison between the vehicle and the ISIS 147768 group (Log rank $p = 0.6482$, Wilcoxon $p = 0.6580$), all tests of trend, homogeneity, and pairwise comparisons are highly statistically significant (all seven Logrank $p \leq 0.0001$, all seven Wilcoxon $p \leq 0.0005$).

Results in female rats are not quite as clear or simple as in the male rats. One might note that the log rank tests places greater weight on later events, while the Wilcoxon test tends to weight them more equally, and thus places more weight on differences earlier in the study than does the log rank test. From Figure A.1.2, the high dose, Group 4, has the lowest survival, particularly later in the study. The PBS vehicle group, Group 1, has the highest or close to highest survival. The survival curves for the other groups were more or less intertwined between curves for these two groups. These differences are sufficient to result in statistically significant tests leading to rejection of the hypothesis of overall homogeneity over all five dose groups, Groups 1-5 (Logrank $p = 0.0022$, Wilcoxon $p = 0.0114$), and homogeneity over the treated groups, i.e. Groups 1-4 (Logrank $p = 0.0006$, Wilcoxon $p = 0.0060$). Results in tests of homogeneity over the Vehicle to Medium dose groups, Groups 1-3, were much more equivocal (Logrank $p = 0.0489$, Wilcoxon $p = 0.2924$), with weak evidence of later differences among these groups. The lower survival in the High dose group is sufficient to result in a statistically significant test in Groups 1-4 (Logrank $p = 0.0003$, Wilcoxon $p = 0.0023$), but there is no evidence of a dose related trend in Groups 1-3 (Logrank $p = 0.1702$, Wilcoxon $p = 0.4727$). Pairwise comparisons in survival between the vehicle and the high dose groups were statistically significant (Logrank $p = 0.0023$, Wilcoxon $p = 0.0046$), but pairwise comparisons of vehicle to the medium ISIS 301012 or ISIS 147768 dose groups were not statistically significant (Logrank $p = 0.0802, 0.2447$, Wilcoxon $p = 0.2924, 0.3097$ respectively).

Results in survival in the mouse study are summarized below:

Table 4. Statistical Significances of Tests of Homogeneity and Trend in Survival in the Mouse Study

Hypothesis Tested	Males		Females	
	Log rank	Wilcoxon	Log rank	Wilcoxon
Mouse Homogeneity over Groups 1-6	0.0012	0.0049	< 0.0001	< 0.0001
Homogeneity over Groups 1-4	0.0004	0.0020	0.0004	0.0026
No trend over Groups 1-4	< 0.0001	0.0003	< 0.0001	0.0044
Homogeneity over Groups 1-3	0.6839	0.6495	0.3498	0.8726
No trend over Groups 1-3	0.4149	0.3829	0.1481	0.6430
No Difference Between Groups 1 vs 3	0.4941	0.4642	0.2108	0.7133
No Difference Between Groups 1 vs 4	0.0084	0.0020	0.0013	0.0520
No Difference Between Groups 1 vs 5	0.7180	0.5746	0.8516	0.5058
No Difference Between Groups 1 vs 6	0.7271	0.8703	0.1329	0.0244
No Difference Between Groups 3 vs 5	0.7793	0.8631	0.1593	0.1592

Figures A.1.3 through A.1.4 in Appendix 1, display survival curves for each mouse gender. From Figure A.1.3, in male mice, the High dose group, Group 4, has the lowest survival, with the other dose groups largely intertwined. These differences are sufficient to result in statistically significant tests of overall homogeneity over all six dose groups, i.e., Groups 1-6, (Logrank $p = 0.0012$, Wilcoxon $p = 0.0049$). The low survival in the High dose groups was sufficient to result in a statistically significant test of homogeneity over the more comparable Vehicle to High dose groups, Groups 1-4, (Logrank $p = 0.0004$, Wilcoxon $p = 0.0020$) as well as a statistically significant test of trend in survival over these four groups (Logrank $p < 0.0001$, Wilcoxon $p = 0.0003$). Pairwise comparisons between the vehicle and the High dose group were statistically significant (Logrank $p = 0.0084$, Wilcoxon $p = 0.0020$). None of the other six tests or comparisons in survival above were statistically significant (all Logrank $p \geq 0.4149$, Wilcoxon $p \geq 0.3829$). Note that the comparison between dose groups 3 and 5 reflects roughly the same monthly dose, but one delivered over four weeks, the other in a single bolus.

From Figure A.1.4, in female mice, by the end of the study the High dose group has the lowest survival, though at first the Vehicle group has the lowest survival. At the end of the study, the ISIS 147764 dose group has the highest survival, with the other dose groups largely intertwined. These differences are sufficient to result in statistically significant tests of overall homogeneity over all six dose groups, Groups 1-6 (both Logrank and Wilcoxon $p < 0.0001$), as well as the test of trend over the four treated groups, Groups 1-4, (Logrank $p < 0.0001$, Wilcoxon $p = 0.0044$). Pairwise comparisons in survival between the vehicle and the high dose groups were statistically significant or close to significance (Logrank $p = 0.0013$, Wilcoxon $p = 0.0520$), while the comparison to ISIS 147764 was equivocal (Logrank $p = 0.1329$, Wilcoxon $p = 0.0244$). But recall that the log rank test emphasizes later differences, while the Wilcoxon test is more sensitive to earlier differences. None of the other six tests above were statistically significant (Logrank $p \geq 0.1593$, Wilcoxon $p \geq 0.1592$).

Of course in a carcinogenicity study, primary interest is on the occurrence of cancers. The statistical analysis of tumors compares tumor incidence over dose groups. Complete tumor incidence tables for each organ listed by the Sponsor and those combined by this reviewer are provided in Tables A.2.3 through A.2.6 in Appendix 3. Tables 5 and 6, displayed below, show those organ tumor combinations that had at least one test of trend or pairwise difference from control that was statistically significant at the usual 0.05 level. For each species by gender by organ the number of animals analyzed and used in the statistical tests is presented first. The tumor incidence for each organ is presented next, with the significance levels of the tests of trend, and the results of pairwise tests between the high, medium, and low dose groups. These statistical tests are supposed to be conditioned on the animals actually evaluated, ignoring those not analyzed. In other words, animals not analyzed are treated as being not at risk. Note that Section 2.2 below discusses some concerns about this data.

To adjust for the multiplicity of tests the so-called Haseman-Lin-Rahman rules discussed in Section 1.3.1.5, below, are often applied. That is, when testing for trend in carcinogenicity over dose and the difference between the highest dose group with a control group, to control the overall Type I error rate to roughly 10% for a standard two species, two sex study, one compares

the unadjusted significance level of the trend test to 0.005 for common tumors (incidence > 1%) and 0.025 for rare tumors, and the pairwise test to 0.01 for common tumors and 0.05 for rare tumors. As also discussed in section 1.3.1.4, employing these adjustments for other tests, like the trend over the PBS vehicle, low, and medium dose groups (i.e. Groups 1, 2, and 3) and the pairwise comparisons between the vehicle and groups other than the high ISIS 301012 dose group can be expected to increase the overall type I error rate to some value above the nominal rough 10% level, possibly considerably higher than this nominal rate.

The following tables display those organ tumor combinations that had at least one comparison statistically significant at the “usual” 0.05 level (but not adjusted for multiplicity). Recall that “PBS” denotes the vehicle, while in this case “I14” denotes the ISIS 147768 dose group in rats and the ISIS 147764 dose group in mice. The trend tests are conducted over both ISIS 301012, PBS to High groups (i.e. Groups 1-4) and a test of trend deleting the High dose (i.e. Groups 1-3). So strictly speaking, the original Haseman-Lin-Rahman rules are most appropriate to the test results denoted “trnd1-4” and “4vs1” in both genders in both species. As discussed in section 1.3.1.3 below, the adjusted number at risk seems to be a more appropriate denominator than the number evaluated when assessing tumor rates.

Table 5. Potentially Statistically Significant Neoplasms in Male Rats

organ tumor	Incidence					Significance levels			
	PBS	ow	Med	Hi	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1	2vs1
Male Rats									
pituitary gland									
# Evaluated	60	60	60	60	60				
Adjusted # at risk	51.3	54.5	43.5	23.5	51.3				
ADENOMA, PARS DISTALIS	36	47	29	13	36	.9782	.9253	.7103	.0331
						.7910	.5859		
skin, subcutis									
# Evaluated	60	60	60	60	60				
Adjusted # at risk	46.0	42.4	32.3	16.4	44.9				
FIBROUS HISTIOCYTOMA	0	1	3	3	1	.0046	.0148	.0652	.4713
						.0301	.4889		
Adjusted # at risk	46.1	41.5	32.7	17.1	46.4				
Fibroma/Fibrosarcoma/Fibrous Histiocytoma	1	6	5	4	4	.0245	.0165	.0396	.0423
						.0490	.1805		

Using the incidence in the PBS vehicle control group to specify whether a tumor is treated as common or rare (i.e., more or less than 1%), in male rats, the tests of trend in fibrous histiocytoma, of the subcutis, over Groups 1-4 is statistically significant ($p = 0.0046 < 0.025$), and, accepting the increase in Type I error from including this test, the test of trend over Groups 1-3 is not quite statistically significant ($p = 0.0301 > 0.025$), nor is either test of trend in pooled fibroma, fibrosarcoma, and fibrous histiocytoma ($p = 0.0245, 0.0490 > 0.005$, respectively). For this pooled tumor the pairwise comparison between the high dose and the vehicle is also not quite statistically significant ($p = 0.0165 > 0.01$). However, the test of differences between the high dose group and vehicle (i.e., Groups 4 versus 1) in fibrous histiocytoma of the subcutis is statistically significant ($p = 0.0148 < 0.05$). The test comparing the low dose to vehicle (i.e. Groups 2 versus 1) in pars distalis adenoma of the pituitary is not statistically significant ($p =$

0.0331 > 0.01). These results illustrate the effect of the Haseman-Lin-Rahman adjustment for multiplicity.

The following tables present similar results for female rats, and later mice. Note that the period ‘.’ in the following tables denotes the p-values of tests of dose groups with none of the particular tumors the specified groups.

Table 6. Potentially Statistically Significant Neoplasms in Female Rats

organ tumor	Incidence					Significance levels			
	PBS	Low	Med	Hi	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1	2vs1
Uterus/Cervix/Vagina									
# Evaluated	60	60	60	60	60				
Adjusted # at risk	42.2	34.2	38.9	31.1	38.8				
GRANULAR CELL TUMOR	4	2	6	10	4	.0016	.0164	.3057	.8437
						.1537	.5858		
ovaries									
# Evaluated	60	60	60	60	60				
Adjusted # at risk	41.8	33.8	37.1	28.6	38.6				
GRANULOSA CELL TUMOR	0	0	0	2	1	.0394	.1611	.	.
						.	.4810		
pituitary gland									
# Evaluated	60	60	60	60	60				
Adjusted # at risk	54.9	54.4	52.9	41.1	55.4				
ADENOMA, PARS DISTALIS	43	51	45	27	50	.9964	.9592	.2463	.0210
						.2873	.0812		
skin, subcutis									
# Evaluated	60	60	60	60	60				
Adjusted # at risk	41.8	34.0	38.1	29.6	38.5				
FIBROSARCOMA	0	1	4	5	1	.0046	.0098	.0491	.4533
						.0215	.4810		
Adjusted # at risk	42.4	34.2	38.9	30.0	38.5				
Fibroma/Fibrosarcoma	1	2	6	6	1	.0103	.0183	.0405	.4203
						.0187	.7275		
Adjusted # at risk	41.8	33.8	38.3	29.8	38.2				
FIBROUS HISTIOCYTOMA	0	0	3	4	0	.0053	.0259	.1067	.
						.0370	.		
Adjusted # at risk	42.4	34.2	40.2	32.4	38.5				
Fibroma/Fibrosarcoma/Fibr. Histiocytoma	1	2	9	10	1	.0003	.0007	.0058	.4203
						.0016	.7275		
thyroid gland									
# Evaluated	60	60	60	60	60				
Adjusted # at risk	42.5	35.3	41.6	28.5	38.8				
C-Cell Adenoma/Carcinoma	2	5	9	3	7	.3373	.3122	.0218	.1474
						.0183	.0564		
uterus with cervix									
# Evaluated	60	60	60	60	60				
Adjusted # at risk	41.8	33.9	38.1	30.5	38.2				
GRANULAR CELL TUMOR	2	1	3	8	1	.0008	.0117	.4635	.8356
						.2910	.8652		

In female rats, the tests of trend in granular cell tumor in both the uterus with cervix and adding the vagina, over the four ISIS 301012 treatment groups including vehicle, (i.e. Groups 1-4) were statistically significant (p = 0.0008, 0.0016 < 0.005, respectively). Similarly the

equivalent tests of trend in these groups in fibrosarcoma and fibrous histiocytoma in the subcutis were also statistically significant ($p = 0.0046, 0.0053 < 0.025$), as was the test of trend for fibrosarcoma over the first three doses ($p = 0.0215 < 0.025$). Further, in the subcutis, the tests of trend in pooled fibroma, fibrosarcoma, and fibrous histiocytoma were statistically significant over both groups 1-4 and 1-3 ($p = 0.0003, 0.0016 < 0.005$, respectively). The test of trend in pooled fibroma and fibrosarcoma was not statistically significant over either groups 1-4 and 1-3 ($p = 0.0103, 0.0187 > 0.005$, respectively). For pairwise tests, again accepting the increase in Type I error, the differences between the high and medium dose groups and the control in fibrosarcoma in the subcutis would be statistically significant ($p = 0.0098, 0.0491 < 0.05$, respectively). In the subcutis the pairwise comparison between the high dose and control in fibrous histiocytoma would also be statistically significant ($p = 0.0259 < 0.05$). The pairwise comparisons between the high and medium dose groups with vehicle in terms pooled fibroma, fibrosarcoma, and fibrous histiocytoma of the subcutis were statistically significant ($p = 0.0007, 0.0058 < 0.01$, respectively). Finally, in the uterus with cervix the comparison between the high dose and control in granular cell tumor was close to statistical significance ($p = 0.0117 \approx 0.01$).

Table 7. Potentially Statistically Significant Neoplasms in Male Mice

organ tumor	Incidence						Significance levels			
	PBS	Low	Med	Hi	Mnth	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1/ 5vs3	2vs1/ 6vs1
Systemic										
# Evaluated	70	70	70	70	70	70				
Adjusted # at risk	50.4	51.3	48.2	37.4	49.0	49.4				
LYMPHOMA	1	5	3	3	9	0	.2800	.2042	.2933	.1069
							.3436	.0065	.0601	1
liver										
# Evaluated	70	70	70	70	70	70				
Adjusted # at risk	49.5	49.8	49.5	36.0	46.4	51.0				
CARCINOMA, HEPATO- CELLULAR	1	2	8	2	1	4	.2512	.3747	.0153	.5000
							.0038	.7366	.9983	.1938
skin, subcutis										
# Evaluated	70	70	70	70	70	70				
Adjusted # at risk	49.4	49.7	47.2	36.9	46.5	50.5				
FIBROSARCOMA	0	0	1	4	0	3	.0021	.0291	.4896	.
							.3241	.	1	.1250
Adjusted # at risk	50.4	49.7	47.2	37.2	46.5	50.5				
Sarcoma/Fibro-/Lipo-	2	0	1	5	0	3	.0072	.1130	.8671	1
							.6913	1	1	.5000
testes										
# Evaluated	70	69	70	70	70	70				
Adjusted # at risk	49.4	48.8	47.7	35.0	46.6	49.4				
ADENOMA, INTERSTITIAL CELL	0	2	4	0	2	1	.6464	.	.0537	.2423
							.0324	.2318	.8933	.5000

In male mice the test of trend over treated Groups 1-4 in fibrosarcoma of the subcutis was statistically significant ($p = 0.0021 < 0.025$) as was the pairwise comparison between the high dose and control ($p = 0.0291 < 0.05$). However the test of trend in pooled sarcoma, fibrosarcoma, and liposarcoma would not be quite statistically significant ($p = 0.0072 > 0.005$). Although the test of trend over the four 301012 groups in hepatocellular carcinoma of the liver would not be classified as statistically significant, accepting the inflation of overall type I error, the corresponding test of trend over the first three groups would be ($p = 0.0038 < 0.005$).

Differences between the monthly dose and vehicle in systemic lymphoma could be attributed to differences in drug or dosing schedule, but accepting the inflation in Type I error, it would be classified as statistically significant ($p = 0.0065 < 0.01$). No other tests or comparisons achieved the Haseman-Lin-Rahman multiplicity adjusted levels of significance, although the test of trend over the first three doses and difference between the medium dose and vehicle in interstitial cell adenoma of the testes would both be close ($p = 0.0324 > 0.025$, $p = 0.0537 \approx 0.05$, respectively).

Table 8. Potentially Statistically Significant Neoplasms in Female Mice

organ tumor	Incidence					I14	Significance levels			
	PBS	Low	Med	Hi	Mnth		trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1/ 5vs3	2vs1/ 6vs1
Systemic										
# Evaluated	70	70	70	70	70	70				
Adjusted # at risk	43.1	44.6	42.4	33.9	49.9	53.2				
HEMANGIOSARCOMA	2	8	6	11	9	2	.0025	.0012	.1250	.0482
							.2073	.0416	.4075	.7660
Adjusted # at risk	43.9	44.6	42.7	34.6	49.9	53.2				
Hemangioma/-sarcoma	3	8	7	12	9	4	.0024	.0022	.1471	.1050
							.1879	.0940	.5272	.6163
harderian glands										
# Evaluated	70	70	68	70	70	70				
Adjusted # at risk	42.3	43.6	41.0	29.9	46.8	54.4				
ADENOMA	1	3	3	4	4	3	.0547	.0857	.2988	.3169
							.2360	.2099	.5649	.4088
liver										
# Evaluated	70	70	68	67	70	69				
Adjusted # at risk	42.9	43.0	41.9	32.2	47.3	55.7				
ADENOMA, HEPATOCELLULAR	4	0	5	11	5	23	<0.0001	.0095	.4844	1
							.1623	.5722	.7148	.0003
Adjusted # at risk	42.3	43.0	40.1	27.5	46.2	53.1				
CARCINOMA, HEPATO-CELLULAR	0	0	0	2	0	2	.0306	.1496	.	.
						3086
Adjusted # at risk	42.9	43.0	41.9	32.6	47.3	56.0				
Hepato. Adenoma/-carc.	4	0	5	12	5	25	<0.0001	.0044	.4844	1
							.1623	.5722	.7148	.0001
lung										
# Evaluated	70	70	70	70	70	70				
Adjusted # at risk	44.3	45.6	45.6	33.7	49.4	54.2				
ADENOMA, BRONCH. ALV.	10	9	17	11	9	8	.0955	.2185	.0942	.7163
							.0346	.7818	.9905	.8974

In female mice, the tests of trend over Groups 1-4 in systemic hemangiosarcoma and pooled hemangioma and hemangiosarcoma were both statistically significant ($p=0.0025$, $0.0024 < 0.005$), as were the similar tests of trend in hepatocellular adenoma and pooled hepatocellular adenoma and carcinoma of the liver (both $p < 0.0001 < 0.005$). Pairwise comparisons between the high dose and PBS vehicle in systemic hemangiosarcoma and pooled hemangioma and hemangiosarcoma were also both statistically significant ($p=0.0012$, $0.0022 < 0.005$), as was the similar comparison in hepatocellular adenoma and pooled hepatocellular adenoma of the liver and carcinoma ($p = 0.0095$, $0.0044 < 0.01$). The comparisons between ISIS 147764 and PBS vehicle in hepatocellular adenoma and pooled hepatocellular adenoma and carcinoma of the liver would also be classified as statistically significant ($p = 0.0003$, $0.0001 < 0.01$). Again, no other comparisons met the multiplicity/rarity adjusted test significance levels.

Complete incidence tables in both species are provided in tables A.2.3 through A.2.6 in Appendix 3, but for reasons of space, do not contain the adjusted number at risk.

1.2. Brief Overview of the Studies

This submission had a rat study (b) (4) study # 1213-003):

Study GT-348-TX-2: 2-Year Subcutaneous Carcinogenicity Study of ISIS 301012 in Sprague-Dawley Rats,

and a fairly similar, mouse study (b) (4) study # 1213-002):

Study GT-348-TX-1: 2-Year Subcutaneous Carcinogenicity Study of ISIS 301012 and ISIS 147764 in CD-1 Mice.

Both studies were conducted (b) (4). Fairly detailed descriptions of these studies are available in Sections 3.2.1 and 3.2.2, below.

1.3. Statistical Issues and Findings

1.3.1. Statistical Issues

In this section, several issues, typical of statistical analyses of these studies, are considered. These issues include details on the survival analyses, tests on tumorigenicity, multiplicity of tests on neoplasms, and the validity of the designs.

1.3.1.1. Survival Analysis:

The survival analyses presented here are based on both the log rank test and the Wilcoxon test comparing survival curves. The log rank tests tend to put higher weight on later events, while the Wilcoxon test tends to weight events more equally, and thus is more sensitive to earlier differences in survival. The logrank test is most powerful when the survival curves track each other, and thus the hazards, i.e., the conditional probability of the event in the next infinitesimal interval, would be roughly proportional. This is the test used by the Sponsor. In the FDA analysis, both tests were used to test both homogeneity of survival among the treatment groups and the effect of dose on trend in survival. Appendix 1 reviews the specific animal survival analyses in more detail. The results of the Sponsor's analysis are summarized in Sections 3.2.1.1 and 3.2.2.1.

1.3.1.2. Multiplicity of Tests on Survival:

Using both the logrank and Wilcoxon tests, for each gender in rats there are 16 tests of survival differences. In rats there are 8 log rank and 8 Wilcoxon tests of survival in each gender, 10 in mice. Assuming tests within each set were independent, but that there actually were no differences in survival across groups, the probability of at least one statistically significant result, at the usual 0.05 level, for each test and for each gender is about 0.34 in rats, and about 0.40 in mice. If we assume the logrank and Wilcoxon tests are independent, which they clearly are not,

these probabilities increase to .56 in rats and .64 in mice. Finally, again, with no differences in survival, the probabilities of such a result in at least one gender increases to .81 in rats and .87 in mice. While the assumptions of independence above are clearly false, this can give some idea of the possible price paid for the multiplicity of hypothesis tests in the statistical frequentist paradigm.

1.3.1.3. Tests on Neoplasms:

The Sponsor's analyses use Fisher exact tests, Cochran-Armitage tests of trend, Peto analyses of neoplasms, and Poly 3 tests. The Cochran-Armitage test is designed to test for trend or pairwise differences in event incidence, weighted by dose. However it ignores differential mortality across dose groups. Inspecting a large number of studies, Bailer and Portier (1988) noted that survival time seemed to fit a Weibull distribution, generally with a shape parameter of between 1 and 5, with 3 a typical value. With t_{\max} denoting the maximal time to terminal sacrifice and t_{obs} the time to death of the animal, they propose weighting the animal by $(t_{\text{obs}}/t_{\max})^k$, so that an animal that survives for say 52 weeks in 104 week study without the tumor being analyzed is counted as $(1/2)^k$ of an animal. For $k = 3$, that means that animal would count as 1/8 of an animal. Further, the $k = 3$ seems to represent tumor incidence where some animals are perhaps more sensitive and respond earlier to the insult than the remaining animals. Under this structure time to incidence would tend to follow a cubic expression. Thus animal with the specific tumor being studied or who survive to terminal sacrifice without the tumor get a weight of 1 when counting the number of animals at risk. However, animals that die early without the tumor are down weighted when counting the number of animals in the risk set for that specific tumor. With differential mortality this can mean a substantial reduction in the size of that risk set. In those tables where a potentially statistically significant result is obtained (i.e., at least one $p \leq 0.05$), the effective size of the risk set for each tumor is listed in the row labeled "Adjusted # at risk", and is thus more appropriate for comparing incidence rates than the unadjusted number evaluated.

Note that the report of the Society of Toxicological Pathology "town hall" meeting in June 2001 recommended the use of this poly-k modification of the Cochran-Armitage tests of trend over the corresponding Peto tests. It is not clear if the Sponsor's poly-3 tests use asymptotic methods or exact test procedures (i.e. based on the permutation distribution) as used in the FDA analysis. Section 2.2, below, discuss some problems inherent in the interpretation of results using this data.

1.3.1.4. Multiplicity of Tests on Neoplasms:

Frequentist hypothesis testing involves accepting or rejecting hypotheses about the parameters of interest on the basis of the values of some statistic. If one does not provide some sort of multiplicity adjustment to the significance level, the chances of rejecting one or more true null hypothesis increases as the number of such tests increases. To avoid this, it is common to adjust for multiplicity in hypothesis testing resulting in an adjustment in experiment-wise Type I error (i.e., the probability of rejecting a true null hypothesis and thus concluding there is an effect when in fact there is none). Based on his extensive experience with such carcinogenicity analyses in standard laboratory rodents, for pairwise tests between the high dose

group and controls in two species, Haseman (1983) claimed that for a roughly 0.10 (10%) overall false positive error rate, rare tumors should be tested at a 0.05 (5%) level, and common tumors (with a historical control incidence greater than 1%) at a 0.01 level. Similarly, Lin and Rahman (1998) showed that tests of trend over all dose groups should be tested at a 0.025 level for rare tumors and 0.005 for common tumors. This approach is intended to balance both Type I error and Type II error (i.e., the error of concluding there is no evidence of a relation to tumorigenicity when there actually is such a relation). Because of possibility of genetic drift, or differences in treatment, in this study the vehicle group is used to determine if the tumor is classified as rare or common.

Significance levels of the pairwise tests between the vehicle group and other dose groups plus tests of trend over more limited dose groups, as well as a comparison between dose groups 3 and 5 in mice (i.e. comparing a monthly dose of 80 mg/kg compared to weekly doses of 20 mg/kg) are also provided. Including these tests can be expected to increase the overall type I error rate to some level above the rough 10% level associated with the Haseman-Lin-Rahman multiplicity adjustment cited above, possibly considerably larger.

1.3.1.5. Validity of the Designs:

When determining the validity of designs there are two key points:

- 1) adequate drug exposure,
- 2) tumor challenge to the tested animals.

1) is related to whether or not sufficient animals survived long enough to be at risk of forming late-developing tumors and 2) is related to the Maximum Tolerated Dose (MTD), designed to achieve the greatest likelihood of tumorigenicity.

Lin and Ali (2006), quoting work by Haseman, have suggested that in standard laboratory rodent species, a survival rate of about 25 animals, out of 50 or more animals, between weeks 80-90 of a two-year study may be considered a sufficient number of survivors as well as one measure of adequate exposure. Note that as a percentage of high dose group animals that survived to week 91, this criterion is barely met in male mice and not met in female mice or either rat gender (Please see Tables 17 and 18 on pages 23 and 24, and Tables 23 and 24 on page 31 and 32). Like the other comments in this section this requires the expertise of the toxicologist, but may suggest that the MTD was exceeded in at least three of four the species-gender combinations.

The mean weight values and derived differences and ratios in the following table were taken directly from the Sponsor's reports (Rat Table 4, pages 142-145 and 161-164, Mouse Table 4, pages 115-118 and 131-134). The change from baseline in the table below is the simple difference between the means at the specified dates, and thus animals that die are only counted at the study initiation, not at the end of the study. The weeks were chosen to be as close to the end of the study, yet having most dose groups with animals still surviving. Recall that the CRO sacrificed all remaining animals when the number of survivors fell too low.

Table 9. Mean Weights and Changes (in g) in Male Rats

Dose Group	Dose mg/kg/period	Week		Change from baseline	% change relative to vehicle	Week 92	Change from baseline	% change relative to vehicle
		1	72					
1. Vehicle	0	289.7	810.1	522.4		810.1	513.3	
2. Low	3	287.5	797.5	510.0	97.6%	797.5	500.4	97.5%
3. Medium	10	286.9	713.3	426.4	81.6%	713.3	346.4	67.5%
4. High	30/25/20 ^c	288.1	576.0	287.9	55.1%	NA	NA	NA
5. I47768	10	285.5	742.4	456.9	87.5%	742.4	456.9	84.4%

Table 10. Mean Weights and Changes (in g) in Female Rats

Dose Group	Dose mg/kg/period	Week		Change from baseline	% change relative to vehicle
		1	88		
1. Vehicle	0	203.7	498.4	294.7	
2. Low	3	205.2	487.8	282.6	95.9%
3. Medium	10	204.7	430.5	225.8	76.6%
4. High	30/25/20 ^c	207.2	342.8	135.6	46.0%
5. I47768	10	208.8	476.1	267.3	90.7%

Table 11. Mean Weights and Changes (in g) in Male Mice

Dose Group	Dose mg/kg/period	Week		Change from baseline	% change relative to vehicle
		1	96		
1. Vehicle	0 weekly	31.0	45.0	14.0	
2. Low	5 weekly	31.0	43.4	14.4	102.9%
3. Medium	20 weekly	31.2	44.2	13.0	92.9%
4. High	60 weekly	31.3	46.7	15.4	110.0%
5. Monthly	80 monthly	31.7	44.8	13.1	93.6%
6. I47764	60 weekly	31.2	43.7	12.5	89.3%

Table 12. Mean Weights and Changes (in g) in Female Mice

Dose Group	Dose mg/kg/period	Week		Change from baseline	% change relative to vehicle	Week 100	Change from baseline	% change relative to vehicle
		1	84					
1. Vehicle	0 weekly	24.6	37.2	12.6		38.2	13.6	
2. Low	5 weekly	24.8	38.7	13.9	110.3%	40.3	15.5	114.0%
3. Medium	20 weekly	25.0	41.2	16.2	128.6%	40.8	15.8	116.2%
4. High	60 weekly	24.8	40.2	15.4	122.2%	NA	NA	NA
5. Monthly	80 monthly	25.3	38.1	12.8	101.6%	38.1	12.9	94.1%
6. I47764	60 weekly	25.0	39.1	14.1	111.9%	40.0	15.0	110.3%

Chu, Ceuto, and Ward (1981), citing earlier work by Sontag *et al* (1976) recommend that the MTD “is taken as ‘the highest dose that causes no more than a 10% weight decrement as compared to the appropriate control groups, and does not produce mortality, clinical signs of toxicity, or pathologic lesions (other than those that may be related to a neoplastic response) that

would be predicted to shorten the animal's natural life span' ” From Table 9 and 10 above, the weight decrement criterion is clearly exceeded in both the medium and high dose groups in both genders in rats, but is not exceeded in either gender in mice. Again, although this requires the expertise of the toxicologist, this may be evidence that the MTD was exceeded in rats, but not in mice.

The Sponsor summarizes food consumption during the rat study as follows: “Slight treatment-related decreases in food consumption (grams per animal per day) were noted during the study when compared to controls, correlating with the dose-dependent mean body weight decreases noted. Statistical significance was variably noted among the treated groups, but was most often reached at the high dose of ISIS 301012 in both sexes (30/25/20 mg/kg/week ISIS 301012 for males and 25/20 mg/kg/week ISIS 301012 for females).” (pages 37-38 of rat report)

According to the Sponsor, in mice: “No clear treatment-related effects on food consumption were noted during the study. While very sporadic statistically significant increases in food consumption among treated groups were occasionally noted when compared to the control group during the study, there was no correlation to the body weight changes noted and no definitive dose-response pattern was evident.” (page 33 of mouse report)

Again from 2) above, excess mortality not associated with any tumor or sacrifice in the higher dose groups might suggest that the MTD was exceeded. This suggests that a useful way to assess whether or not the MTD was achieved is to measure early mortality not associated with any identified tumor. If this is high in the higher dose groups it suggests that animals tend to die before having time to develop tumors. Tables 9 and 10, below, display the number of animals in each dose group that died of a natural death or moribund sacrifice, but did not show any tumors (i.e., the “Event”):

Table 13. Natural Death with No Identified Tumor in Rats (Male/Female)

		1. Vehicle	2. Low	3. Medium	4. High	5. I47768
Males	Event	55	57	50	31	53
	No event	5	3	10	29	7
Females	Event	60	59	59	51	59
	No event	0	1	1	9	1

It appears that in male rats there is strong evidence of a dose related trend over the four ISIS 301012 treatment groups (with vehicle). Although the trend is apparent, this result is confirmed by the chi-square test of homogeneity ($p < 0.0001$). There are two few events in the no event category in female rats for the validity of the chi-square test, but the large number of deaths without tumor in the high dose group is sufficient to lead to a significant test rejecting homogeneity using Fisher's exact test ($p < 0.0003$). Event time adjusted log rank and Wilcoxon tests were also statistically significant in both genders.

Table 14. Natural Death with No Identified Tumor in Mice (Male/Female)

		1. Vehicle	2. Low	3. Medium	4. High	5. Monthly	6. I47764
Males	Event	51	50	51	46	51	52
	No event	19	20	19	24	19	18
Females	Event	52	53	50	46	56	57
	No event	18	17	20	24	14	13

Results in mice are much less clear. The chi-square tests of homogeneity were not statistically significant (Males $p = 0.9005$, Females $p = 0.2912$). However tests of differences in time to event were statistically significant in females (Logrank $p = 0.0155$, Wilcoxon $p = 0.0292$), but not in males.

Thus, in both genders in rats there is evidence that the incidence of a dose related lack of homogeneity in early death without tumors. With the evident trend over the four ISIS 301012 treatment groups, this may suggest that the MTD was exceeded in both genders in rats. In mice results are much less clear. In male mice this particular test provides no evidence that the MTD was exceeded, but there is some evidence it was exceeded in female mice, although this evidence is weaker than in rats.

Once again, like the other observations above, these require the expertise of the toxicologist, but these tests do not seem to provide evidence that the MTD may have been exceeded in rats and perhaps in mice..

1.3.2. Statistical Findings

Please see Section 1.1 above.

2. INTRODUCTION

2.1. Overview

This submission summarizes the results of two year rat and mouse studies to assess the carcinogenic potential of the potential carcinogenicity of the test article, ISIS 301012 (human-specific apoB inhibitor), with weekly dosing by subcutaneous injection. Both studies were actually conducted, starting in 2008, by (b) (4)

2.2. Data Sources

The Sponsor provided a number of SAS transport files. For both rats and mice these were labeled as:

food.xpt	micro.xpt	signs.xpt	weights.xpt
hemat.xpt	mortal.f.xpt	tumor.f.xpt	
macro.xpt	mortal.m.xpt	tumor.m.xpt	

In addition the rat study had transport files organwtm.xpt and organwtf.xpt, for males and females, respectively. In the mouse study these were combined into a single file, organwt.xpt. Each transport file contained a single SAS data set related to the name associated with the

transport file, and usually named the same.

The tumor data set requested by the biostatistics group has three kinds of records. One record per animal is needed for each organ tumor combination, plus another record for each organ not examined, with at least one record per animal. The set of animals at risk for developing tumors in each organ is supposed to be reduced for each animal where the organ was not examined. These not examined organ tumor records were missing from the tumor analysis data set. If one studies the Individual Animal Data Record in the Pathology section for each study, it is apparent that the some organs in a number of animals were not examined, sometimes due to autolysis, sometimes just not examined. But these were not reflected in the tumor data set provided. Finally, each animal is to have at least one record. So the submitted data set only included the first and last kinds of records.

The micro data set listed above did include records indicating that some organs were not examined. After fixing some inconsistencies in dose group labeling, and organ and tumor names, the information from these records was incorporated into the tumor data set. This did require matching character strings across data sets and may have introduced some error (for example, “is ” and “ is” are different strings!). Further, it is not clear if the information in the micro data set was exhaustive, and covered all organ tumor combinations. Since it is not clear if all animals not examined for all tumors are included in the micro data set, it is recommended that the tumor incidence responses be interpreted as the joint response to two assumed random processes, choice of organ to examine AND display tumor development, not merely the latter.

Finally, the tumor data sets were characterized by very fine subdivisions of organs, generally implying few tumors. Statistically it is difficult to distinguish between a small number of events and no events, which makes it difficult to detect any statistical carcinogenicity signal. A number of organs were combined, but the combinations chosen may not be optimal, and clearly too many small tumor counts are included in the incidence tables.

3. STATISTICAL EVALUATION

3.1. Evaluation of Efficacy

NA

3.2. Evaluation of Safety

3.2.1. Study GT-348-TX-2: 2-Year Subcutaneous Carcinogenicity Study of ISIS 301012 in Sprague-Dawley Rats,

STUDY DURATION: 104 Weeks (planned): Group 4 (High dose) Males necropsied at week 74
Group 3 (Medium dose) Males necropsied at week 96
Group 4 (High dose) Females necropsied at week 92
Group 2 (Low dose) Females necropsied at week 96
Group 3 (Medium dose) Females necropsied at week 100

EXPERIMENTAL START DATE: 14 January 2008
 EXPERIMENTAL TERMINATION: 13 January 2010
 RAT STRAIN: Sprague Dawley CD® [CrI:CD®(SD)] Rats
 ROUTE: Weekly Subcutaneous Injection

The Sponsor summarizes the study design as follows: “This study was conducted to evaluate the potential carcinogenicity of the test article, ISIS 301012 (human-specific apoB inhibitor), in Sprague-Dawley rats after once weekly treatment administered via subcutaneous injection for up to 2 years. Three treatment groups of 60 male and 60 female CD® [CrI:CD®(SD)] rats were administered ISIS 301012 at dose levels of 3, 10, and 30/25/20 (males) or 25/20 (females) mg/kg/week and one group of 60 animals/sex received the rat-specific analog, ISIS 147768 at a dose level of 10 mg/kg/week. The high doses of ISIS 301012 were adjusted to lower doses when measures of renal function indicated increasing toxicity. One group of 60 animals/sex served as the control and received the vehicle, phosphate buffered saline (PBS).” (page 11 of rat report)

The following table (a repeat of Table 1 above) illustrates this further:

Table 15. Design of Rat Study (60 animals per main study group/gender)

Treatment Group	Vehicle or Test Article	Male Dosage (mg/kg)	Dose Volume (mL/kg)	Dosing Interval (weeks) ^a	Female Dosage (mg/kg)	Dose Volume (mL/kg)	Dosing Interval (weeks) ^a
1. Vehicle	PBS	0	5/4.17/2 ^b	1 to 105	0	5/4.17/2 ^b	1 to 105
2. Low	ISIS 301012	3	2	1 to 105	3	2 ^f	1 to 96
3. Medium	ISIS 301012	10	2	1 to 94 ^e	10	2 ^f	1 to 98
4. High	ISIS 301012	30/25/20 ^c	5/4.17/2/1.6 ^d	1 to 70 ^e	25/20 ^a	4.17/2/1.6 ^{df}	1 to 88
5. 147768 ^b	ISIS 147768	10	2	8 to 105	10	2	8 to 105

^a Dosing ceased when survival reached 20 within a sex in a particular group, with terminal necropsy then occurring when survival subsequently reached 15.

^b The dose volume for control animals was reduced to 4.17 mL/kg based on the dose level reduction of Group 4 males during Week 2 and to 2 mL/kg based on the Group 4 test article concentration increase during Week 13.

^c The dose level for male animals was reduced to 25 mg/kg/week during Week 2 and for all animals to 20 mg/kg/week during Week 25.

^d The dose volume for males was reduced to 4.17 mL/kg based on the dose level reduction during Week 2 and for all animals to 2 mL/kg based on the dose concentration increase of 6 mg/mL to 12.5 mg/mL during Week 13 and to 1.6 mL/kg based on the dose level reduction during Week 25.

^e Males: Group 3 were necropsied at Week 96, Group 4 at week 74.

^f Females: Group 2 were necropsied at Week 98, Group 3 at week 100, Group 4 at week 92.

In words: “Three treatment groups of 60 male and 60 female CD® [CrI:CD®(SD)] rats were administered ISIS 301012 at dose levels of 3, 10, and 30/25/20 (males) or 25/20 (females) mg/kg/week and one group of 60 animals/sex received the rat-specific analog, ISIS 147768 at a dose level of 10 mg/kg/week. The high doses of ISIS 301012 were adjusted to lower doses when

measures of renal function indicated increasing toxicity. One group of 60 animals/sex served as the control and received the vehicle, phosphate buffered saline (PBS).” (page 11 of rat report)

“The animals were dosed at designated dose volumes (1.6 to 5 mL/kg) for approximately 2 years, with the exception of the following groups: females at 3 mg/kg/week ISIS 301012 (96 weeks of dosing), males and females at 10 mg/kg/week ISIS 301012 (94 and 98 weeks of dosing, respectively), males at 30/25/20 mg/kg/week ISIS 301012 (70 weeks of dosing), and females at 25/20 mg/kg/week ISIS 301012 (88 weeks of dosing). Dosing was stopped when survival reached 20 within a sex in a particular group, with terminal necropsy when survival reached 15. Dosing for animals receiving ISIS 147768 was initiated during Week 8. An additional group of 25 animals/sex was maintained during the study as sentinel animals.” (page 11 of rat report)

The Sponsor indicates that “a simple randomization procedure” was used to assign animals to the control and treatment groups, but details are not apparent. The Sponsor notes that at randomization males weighed between 220 and 254 g and females between 164 to 201 g. Animals were housed individually with food and water available *ad libitum*, although food was withheld during “designated periods”.

The Sponsor states that the “dose levels (0, 3, 10, and 25/30 mg/kg/week) were selected by the Sponsor on the basis of available pharmacology and toxicology data from previous studies The high dose of 25 or 30 mg/kg/week was considered to be a maximum tolerated dose (MTD) in female and male rats, respectively, for a 2 year study. The MTD was defined by a combination of significant reductions in body weight gain and induction of immune stimulation [from prior ISIS 301012 studies]. The high doses chosen for male and female rats produced roughly 15% decrease in body weight gain in 5 months of treatment.” (page 22 of report) As indicated in Section 1.3.1.5 above, this reviewer finds evidence that the MTD was exceeded in both genders in rats. Of course actual determination of such a conclusion requires the expertise of the toxicologist.

3.2.1.1. Sponsor’s Results and Conclusions

This section will present a summary of the Sponsor’s analysis on survivability and tumorigenicity in rats.

Sponsor’s Survival analysis:

The Sponsor provided the following summary of survival at the end of the study (page 35 of rat report): “The number of study animals surviving to the scheduled terminal necropsy (necropsy count) at Week 105 (Day 731) were as follows (out of 60 animals in each main group):”

Table 16. Sponsor Unnumbered Text (copied from page 35)

The number of animals surviving to the scheduled terminal necropsy (Week 105) ^a		
Dose Level	Male	Female
0 mg/kg/week	24 (40%)	22 (38%)
3 mg/kg/week ISIS 301012	21 (35%)	0 (0%)
10 mg/kg/week ISIS 301012	0 (0%)	0 (0%)
30/25/20 mg/kg/week ISIS 301012 (male) 30/25/20 mg/kg/week ISIS 301012 (male)	0%	0 (0%)
10 mg/kg/week ISIS 147768	22 (38%)	16 (28%)
^a Respective survival percentage calculations include/reflect either death or necropsy at Week 105 of the study for groups surviving to scheduled terminal necropsy . . .		

The report continues: “Thus, the incidence of mortality was higher than controls for males at ≥ 10 mg/kg/week ISIS 301012 and females at ≥ 3 mg/kg/week ISIS 301012. This was considered a treatment related effect. The animals in these respective groups were necropsied early (after consultation with the U.S. FDA), with dosing terminating when survival reached 20, and the animals necropsied when survival reached 15. Therefore, females at 3 mg/kg/week ISIS 301012 underwent 96 weeks of dosing (and were necropsied on Day 681), males and females at 10 mg/kg/week ISIS 301012 underwent 94 and 98 weeks of dosing, respectively (and were necropsied on Days 666 and 697, respectively), while males at 30/25/20 mg/kg/week ISIS 301012 underwent 70 weeks of dosing (and were necropsied on Day 517) and females at 25/20 mg/kg/week ISIS 301012 underwent 88 weeks of dosing (and were necropsied on Day 641).” (page 35 of rat report)

Sponsor’s Tumorigenicity analysis:

The Sponsor reports results of Fisher Exact tests, Cochran-Armitage trend tests, Peto tests, and Poly-k analyses of neoplasms in Table 15 (pages 526-593) of the rat report. They summarize results as follows:

“Neoplastic findings – Males

“There were no statistically significant increases in any type of neoplasm in the male rats with the Fisher Exact test for pair-wise comparisons, the Cochran-Armitage Trend test, the Peto test, or the Onset Rate test.

“ In males, malignant fibrous histiocytoma was statistically significantly increased with the Poly-3 pair-wise comparisons at 10 and 30/25/20 mg/kg/week ISIS 301012 (3/60 or 5.00% overall rate, 9.36% adjusted rate for 10 mg/kg/week ISIS 301012 and 3/60 or 5.00% overall rate, 18.46% adjusted rate for 30/25/20 mg/kg/week ISIS 301012 compared to 0/60 in the control group). The ^{(b) (4)} historical control data for this tumor type in males has a range of 0-2.0% with a mean incidence of 0.4% (rare tumor). Although the increase is slight in terms of raw incidence (the actual incidence in these two groups only exceeds the ^{(b) (4)} historical control raw incidence for a group by 2 tumors), this tumor type may be increased as a result of

the subcutaneous administration of the test article as will be discussed in the neoplastic findings section for females below.

“Also with the Poly-3 pair-wise comparisons in males the incidence of thyroid follicular cell carcinoma was increased at 10 mg/kg/week ISIS 301012 (2/60 or 3.33% overall rate, 6.36% adjusted rate compared to 0/60 in the control group). The (b) (4) historical control data for this tumor type in males has a range of 0-2.9% with a mean incidence of 0.5% (rare tumor). This statistically significant increase was considered incidental and not test article-related as the overall rate was only slightly greater than the historical control range, the concurrent control was at the bottom of the historical control range, the increase was not dose-related, and there was not a similar effect in the females. Further, the combined incidence of follicular cell adenoma and carcinoma was not statistically significant as compare to the control group.

“Finally, the incidence of large granular lymphocyte leukemia was statistically significantly increased in males with the Poly-3 Trend test at 10 mg/kg/week ISIS 147768 (2/60 or 3.33% overall rate, 4.49% adjusted rate compared to 0/60 in the control group). The (b) (4) historical control data for this tumor type in males has a range of 0-1.4% with a mean incidence of 0.5% (rare tumor). This statistically significant increase was considered incidental and not test article-related as the overall rate was only slightly greater than the historical control range, the concurrent control was at the bottom of the historical control range, and there was not a similar effect in the females.

“Neoplastic findings – Females

There were no statistically significant increases in any type of neoplasm in the female rats with either the Fisher Exact test for pair-wise comparisons, the Cochran-Armitage Trend test, the Peto test, the Onset rate test, or the Poly-3 Trend test.

“In females, malignant fibrous histiocytoma was statistically significantly increased with the Poly-3 pair-wise comparisons at 10 and 25/20 mg/kg/week ISIS 301012 (3/60 or 5.00% overall rate, 7.91% adjusted rate for 10 mg/kg/week ISIS 301012 and 4/60 or 6.67% overall rate, 13.58% adjusted rate for 25/20 mg/kg/week ISIS 301012 compared to 0/60 in the control group). The (b) (4) historical control data for this tumor type in females has a range of 0-3.3% with a mean incidence of 0.2% (rare tumor). The increase is slight in terms of raw incidence in both groups, exceeding the (b) (4) historical control raw incidence for a group by only 2 tumors.

“Fibrosarcoma was also statistically significantly increased in females by the Poly-3 pair-wise comparisons at 25/20 mg/kg/week ISIS 301012 (5/60 or 8.33% overall rate, 17.08% adjusted rate compared to 0/60 in the control group). The (b) (4) historical control data for this tumor type in females has a range of 0-4.6% with a mean incidence of 1.1% (common tumor). As noted with the malignant fibrous histiocytoma, the increase in fibrosarcoma is slight in terms of raw incidence, exceeding the (b) (4) historical control raw incidence for a group by only 2 tumors.

“Malignant fibrous histiocytoma (MFH) and fibrosarcoma are malignant mesenchymal neoplasms (sarcomas). Malignant fibrous histiocytomas have variable histologic features, ranging from populations of plump spindle cells exhibiting a characteristic cartwheel or “storiform” pattern with abundant collagen formation to populations of highly pleomorphic spindle cells, multinucleated, and bizarre giant cells with sparse collagen production. Enzyme cytochemical studies of these neoplasms have shown the presence of lysosomal enzyme activity characteristic of tissue histiocytes, while ultrastructural studies have demonstrated features of both fibroblasts and histiocytes as well as a small number of primitive mesenchymal cells.

“Fibrosarcomas are comprised of more monomorphic spindle cells with oval nuclei and basophilic cytoplasm arranged in interlacing fascicles or interwoven in a typical herringbone pattern with variable mitotic activity and collagenous intercellular matrix. Ultrastructural studies have shown the spindle cell to be fibroblastic MFH . . . and fibrosarcomas occur spontaneously in rats, but are also known to arise at the implantation site of inert materials.²⁶ The rodent is known to be particularly susceptible to development of sarcomas in the subcutaneous tissue in the presence of chronic tissue irritation and inflammation²⁷ as occurred at the injection sites in this study (lymphohistiocytic infiltrates, as discussed above). Thus, while the increase in MFH and fibrosarcoma is small in this study, it is possible that this slight increase is secondary repeated subcutaneous injection into a small area.

“Also with the Poly-3 pair-wise comparisons the incidence in females of adenoma of the pars distalis of the pituitary gland was statistically significantly increased in the 3 mg/kg/week ISIS 301012 (51/60 or 85.00% overall rate, 93.75% adjusted rate compared to 43/60 or 71.67% overall rate, 78.41% adjusted rate for the control group). The (b) (4) historical control data for this tumor type has a range of 60.0 to 88.3% with a mean incidence of 76.7% (common tumor). This statistically significant increase was considered incidental and not test article-related as the overall rate was within the historical control range, the increase was not dose related, and the combined incidence of adenoma and carcinoma of the pars distalis of the pituitary was not statistically significant as compared to the control group.

“Finally, the incidence of granular cell tumors of the uterus was statistically significantly increased with the Poly-3 pair-wise comparisons at 25/20 mg/kg/week ISIS 301012 (8/60 or 13.33% overall rate, 26.46% adjusted rate compared to 2/60 or 3.33% overall rate, 4.81% adjusted rate in the control group). The (b) (4) historical control data for this tumor type has a range of 0-10% with a mean incidence of 1.6% (common tumor). Granular cell tumors of the female reproductive tract are comprised of cells that are uniform in appearance with abundant granular eosinophilic cytoplasm with prominent interstitial collagen. Most tumors are small and are only recognized microscopically so the incidence among groups may vary due to the inability to identify the tumors macroscopically. In a report of nine control groups from 104 week studies with (b) (4) Sprague-Dawley rats the incidence of granular cell tumors varied from 3.4 to 13.3%.²⁸ This statistically significant increase in granular cell tumors was considered incidental and not test article-related as the overall rate was only slightly greater than the (b) (4) historical control range, the concurrent

control group was on the low side of the historical control range, and the overall incidence was within the range of that reported for nine control groups from 104 week studies with (b) (4) Sprague-Dawley rats.

“No other tumor type was statistically significant in either sex by any of the statistical tests use[d].” (pages 46-48 of rat report)

3.2.1.2. FDA Reviewer's Results

This section will present the Agency findings on survival and tumorigenicity in male and female rats.

Survival analysis:

The following tables (Table 17 for male rats, Table 18 for females) summarize the mortality results for the study groups. The data were grouped for the specified time period, and present the number of deaths during the time interval over the number at risk at the beginning of the interval. The percentage cited is the percent that survived at the end of the interval. In these tables the terminal period only includes those animals were sacrificed. Male rats that died of other causes during the terminal period are included in the preceding, overlapping time period. The Kaplan-Meier survival plots in Appendix 1 provide a more detailed picture of the profile of mortality losses.

Table 17. Summary of Male Rats Survival (dose label/dose/weeks dosing)

Period (Weeks)	Vehicle 0/ 1-105	Low 3/ 1-105	Medium 10/ 1- 94	High 30/ 1- 70	ISIS 147768 10/1-105
1-52	4/60 ¹ 93% ²	5/60 92%	4/60 93%	8/60 87%	4/60 93%
53-78	8/56 80%	12/55 72%	18/56 63%	37/52 25%	6/56 83%
79-91	6/48 70%	8/43 58%	15/38 38%	0	9/50 68%
92-105	18/42 40%	14/35 35%	9/23 23%	0	19/41 37%
Terminal ³	24	21	14	15	22

¹ number of deaths / number at risk

² overall per cent survival to end of period.

³ number of animals that survived to terminal sacrifice.

Table 18. Summary of Female Rats Survival (dose label/dose/weeks dosing)

Period (Weeks)	Vehicle 0/ 1-105	Low 3/ 1- 96	Medium 10/ 1- 98	High 25/ 1- 88	ISIS 147768 10/ 1-105
1-52	1/60 ¹ 98% ²	7/60 88%	3/60 95%	4/60 93%	2/60 97%
53-78	17/59 70%	11/53 70%	12/57 75%	22/56 57%	15/58 72%
79-91	11/42 52%	13/42 48%	17/45 47%	17/34 28%	16/43 45%
92-105	9/31 37%	14/29 25%	13/28 25%	2/17 25%	11/27 27%
Terminal ³ 105	22	15	15	15	16

¹ number of deaths / number at risk

² overall per cent survival to end of period.

³ number of animals that survived to terminal sacrifice.

Table 19 below provides the significance levels of the tests of homogeneity and trend over dose groups as proposed in Section 1.3.1.1 above (and is a repeat of Table 3 above and Table A.1.1 in Appendix 1)..

Table 19. Statistical Significances of Tests of Homogeneity and Trend in Survival in Rats

Hypothesis Tested	Males		Females	
	Log rank	Wilcoxon	Log rank	Wilcoxon
Rat Homogeneity over Groups 1-5	< 0.0001	< 0.0001	0.0022	0.0114
Homogeneity over Groups 1-4	< 0.0001	< 0.0001	0.0006	0.0060
No trend over Groups 1-4	< 0.0001	< 0.0001	0.0003	0.0023
Homogeneity over Groups 1-3	0.0001	0.0005	0.0489	0.2197
No trend over Groups 1-3	< 0.0001	0.0002	0.1702	0.4727
No Difference Between Groups 1 vs 3	< 0.0001	< 0.0001	0.0802	0.2924
No Difference Between Groups 1 vs 4	< 0.0001	< 0.0001	0.0023	0.0046
No Difference Between Groups 1 vs 5	0.6482	0.6580	0.2447	0.3097

From Figure A.1.1, in Appendix 1, in male rats, at first there is a clear decreasing survival over dose with exception that Group 5, the ISIS 147768 group, closely tracks the group with the highest survival, the vehicle group, Group 1. This is consistent with the results of the tests above. With the exception of the pairwise comparison between the vehicle and the ISIS 147768 group (Log rank $p = 0.6482$, Wilcoxon $p = 0.6580$), all tests of trend, homogeneity, and pairwise comparisons are highly statistically significant (all seven Logrank $p \leq 0.0001$, all Wilcoxon $p \leq 0.0005$).

Survival results in female rats are not quite as clear or simple as in the male rats above. From Figure A.1.2, the high dose, Group 4, has the lowest survival, particularly later in the study. The vehicle group, Group 1, has the highest or close to highest survival. The other groups were more or less intertwined between these two groups. These differences are sufficient

to result in statistically significant tests of overall homogeneity over all five dose groups, Groups 1-5 (Logrank p = 0.0022, Wilcoxon p = 0.0114), and homogeneity over the Vehicle to High dose, Groups 1-4 (Logrank p = 0.0006, Wilcoxon p = 0.0060). Tests of homogeneity over the Vehicle to Medium dose groups, Groups 1-3, were much more equivocal (Logrank p = 0.0489, Wilcoxon p = 0.2924), with weak evidence of later differences among these groups. The lower survival in the High dose group is sufficient to result in a statistically significant in Groups 1-4 (Logrank p = 0.0003, Wilcoxon p = 0.0023), but there is no evidence of a dose related trend in Groups 1-3 (Logrank p = 0.1702, Wilcoxon p = 0.4727). Pairwise comparisons between the vehicle and the high dose groups were statistically significant (Logrank p = 0.0023, Wilcoxon p = 0.0046), but pairwise comparisons to the medium and ISIS 147768 dose groups were not statistically significant (Logrank p = 0.0802, 0.2447, Wilcoxon p = 0.2924, 0.3097, respectively).

Tumorigenicity analysis:

As discussed in Section 1.3.1.5, the Haseman-Lin-Rahman rules for adjusting for multiplicity in a single species study specify that for a very rough 0.10 (10%) overall false positive error rate, both overall trend and the comparison between control and the high dose should be tested at a 0.05 (5%) level in rare tumors (background incidence 1% or less) and at 0.01 (1%) level in common tumors. In this analysis we use the incidence in the PBS vehicle control group to specify whether a tumor is treated as common or rare. Note that the period ‘.’ in the table denotes the p-values of tests of dose groups with none of the particular tumors the specified groups.

The following table 20 is a repeat of tables 5 and 6, above.

Table 20. Potentially Statistically Significant Neoplasms in Rats

Gender/ organ tumor	Incidence					Significance levels			
	PBS	Low	Med	Hi	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1	2vs1
Male Rats									
pituitary gland									
# Evaluated	60	60	60	60	60				
Adjusted # at risk	51.3	54.5	43.5	23.5	51.3				
ADENOMA, PARS DISTALIS	36	47	29	13	36	.9782	.9253	.7103	.0331
						.7910	.5859		
skin, subcutis									
# Evaluated	60	60	60	60	60				
Adjusted # at risk	46.0	42.4	32.3	16.4	44.9				
FIBROUS HISTIOCYTOMA	0	1	3		3	1	.0046	.0148	.0652
							.0301	.4889	.4713
Adjusted # at risk	46.1	41.5	32.7	17.1	46.4				
Fibroma/Fibrosarcoma/Fibrous Histiocytoma	1	6	5	4	4	4	.0245	.0165	.0396
							.0490	.1805	.0423
Female Rats									
Uterus/Cervix/Vagina									
# Evaluated	60	60	60	60	60				
Adjusted # at risk	42.2	34.2	38.9	31.1	38.8				
GRANULAR CELL TUMOR	4	2	6	10	4	4	.0016	.0164	.3057
							.1537	.5858	.8437

Table 20. (cont.) Potentially Statistically Significant Neoplasms in Rats

Gender/ organ tumor	Incidence					Significance levels			
	PBS	Low	Med	Hi	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1	2vs1
Female Rats (cont.)									
ovaries									
# Evaluated	60	60	60	60	60				
Adjusted # at risk	41.8	33.8	37.1	28.6	38.6				
GRANULOSA CELL TUMOR	0	0	0	2	1	.0394	.1611	.	.
						.	.4810		
pituitary gland									
# Evaluated	60	60	60	60	60				
Adjusted # at risk	54.9	54.4	52.9	41.1	55.4				
ADENOMA, PARS DISTALIS	43	51	45	27	50	.9964	.9592	.2463	.0210
						.2873	.0812		
skin, subcutis									
# Evaluated	60	60	60	60	60				
Adjusted # at risk	41.8	34.0	38.1	29.6	38.5				
FIBROSARCOMA	0	1	4	5	1	.0046	.0098	.0491	.4533
						.0215	.4810		
Adjusted # at risk	42.4	34.2	38.9	30.0	38.5				
Fibroma/Fibrosarcoma	1	2	6	6	1	.0103	.0183	.0405	.4203
						.0187	.7275		
Adjusted # at risk	41.8	33.8	38.3	29.8	38.2				
FIBROUS HISTIOCYTOMA	0	0	3	4	0	.0053	.0259	.1067	.
						.0370	.		
Adjusted # at risk	42.4	34.2	40.2	32.4	38.5				
Fibroma/Fibrosarcoma/Fibr. Histiocytoma	1	2	9	10	1	.0003	.0007	.0058	.4203
						.0016	.7275		
thyroid gland									
# Evaluated	60	60	60	60	60				
Adjusted # at risk	42.5	35.3	41.6	28.5	38.8				
C-Cell Adenoma/Carcinoma	2	5	9	3	7	.3373	.3122	.0218	.1474
						.0183	.0564		
uterus with cervix									
# Evaluated	60	60	60	60	60				
Adjusted # at risk	41.8	33.9	38.1	30.5	38.2				
GRANULAR CELL TUMOR	2	1	3	8	1	.0008	.0117	.4635	.8356
						.2910	.8652		

In male rats, the tests of trend in fibrous histiocytoma, of the subcutis, over Groups 1-4 is statistically significant ($p = 0.0046 < 0.025$), and, accepting the increase in Type I error from including this test, the test of trend over Groups 1-3 is not quite statistically significant ($p = 0.0301 > 0.025$), nor is either test of trend in pooled fibroma, fibrosarcoma, and fibrous histiocytoma ($p = 0.0245, 0.0490 > 0.005$, respectively). For this pooled tumor the pairwise comparison between the high dose and the vehicle is also not quite statistically significant ($p = 0.0165 > 0.01$). However, the test of differences between the high dose group and vehicle (i.e., Groups 4 versus 1) in fibrous histiocytoma of the subcutis is statistically significant ($p = 0.0148 < 0.05$). The test comparing the low dose to vehicle (i.e. Groups 2 versus 1) in pars distalis adenoma of the pituitary is not statistically significant ($p = 0.0331 > 0.01$). These results illustrate the effect of the Haseman-Lin-Rahman adjustment for multiplicity.

In female rats, the tests of trend in granular cell tumor in both the uterus with cervix and adding the vagina (to give the combined uterus/cervix/vagina), over the four ISIS 301012 treatment groups including vehicle, (i.e. Groups 1-4) were statistically significant ($p = 0.0008$, $0.0016 < 0.005$, respectively). Similarly the equivalent tests of trend in these groups in fibrosarcoma and fibrous histiocytoma in the subcutis were also statistically significant ($p = 0.0046$, $0.0053 < 0.025$), as was the test of trend for fibrosarcoma over the first three doses ($p = 0.0215 < 0.025$). Further, in the subcutis, the tests of trend in pooled fibroma, fibrosarcoma, and fibrous histiocytoma were statistically significant over both groups 1-4 and 1-3 ($p = 0.0003$, $0.0016 < 0.005$, respectively). The test of trend in pooled fibroma and fibrosarcoma was not statistically significant over either groups 1-4 and 1-3 ($p = 0.0103$, $0.0187 > 0.005$, respectively). For pairwise tests, again accepting the increase in Type I error, the difference between the high and medium dose groups in fibrosarcoma in the subcutis would be statistically significant ($p = 0.0098$, $0.0491 < 0.05$, respectively). In the subcutis the pairwise comparison between the high dose and control in fibrous histiocytoma would also be statistically significant ($p = 0.0259 < 0.05$). The pairwise comparisons between the high and medium dose groups with vehicle in terms pooled fibroma, fibrosarcoma, and fibrous histiocytoma of the subcutis were statistically significant ($p = 0.0007$, $0.0058 < 0.01$, respectively). Finally, in the uterus with cervix the comparison between the high dose and control in granular cell tumor was close to statistical significance ($p = 0.0117 \approx 0.01$).

Complete tumor incidence tables, but without the adjusted number at risk, are provided in tables A.2.3 and A.2.4 of Appendix 3.

3.2.2. Study GT-348-TX-1: 2-Year Subcutaneous Carcinogenicity Study of ISIS 301012 and ISIS 147764 in CD-1 Mice.

STUDY DURATION: 104 Weeks (planned): Group 4 (High dose) Males sacrificed at week 74
Group 4 (High dose) Females necropsied at week 83
Group 3 (Medium dose) Males necropsied at week 98

EXPERIMENTAL START DATE: 1 June 2007

EXPERIMENTAL TERMINATION: 4 June 2009

MOUSE STRAIN: (b) (4) Crl:CD1[®] (Icr) Mice

ROUTE: Weekly Subcutaneous Injection (plus one monthly injection dose group)

The Sponsor notes that “The objective of this study was to evaluate the potential carcinogenicity of ISIS 301012 (human-specific apoB inhibitor) and characterize the potential carcinogenic effects of a mouse specific apoB inhibitor (ISIS 147764) in CD-1 mice after once weekly treatment administered via subcutaneous injection for 2 years. Both ISIS 301012 and ISIS 147764 were evaluated to characterize the potential effects of the compound intended for clinical use (ISIS 301012) as well as the potential effects of continuous apoB inhibition. By examining the effects of the sequence intended for clinical use (ISIS 301012) as well as a murine active sequence, information was obtained on both the potential effects of the chemical class and those attributable to the pharmacologic activity.” (page 16 of mouse report)

The Sponsor states that originally 500 male animals and 501 female animals were acquired [REDACTED] ^{(b) (4)} for this study. Further, the Sponsor notes that “The animals considered suitable for study were weighed, and using a standard, by weight, block randomization procedure, 445 male and 445 female animals (weighing 26.3 to 33.5 g and 21.7 to 26.5 g, respectively, at randomization) were assigned to the control and treatment groups . . .”. (page 19 of report) Gross aspects of the study designs for the main study animals are summarized below (a repeat of table 2):

Table 21. Design of Mouse Study (70 animals per main study group/gender)

Treatment Group	Vehicle or Test Article	Dosage (mg/kg)	Frequency of Dosing	Male Dosing Interval (weeks) ^a	Female Dosing Interval (weeks) ^a
1. Vehicle	PBS	0	Once Weekly	1 to 105	1 to 105
2. Low	ISIS 301012	5	Once Weekly	1 to 105	1 to 105
3. Medium	ISIS 301012	20	Once Weekly	1 to 105	1 to 98 ^b
4. High	ISIS 301012	60	Once Weekly	1 to 95 ^b	1 to 83 ^b
5. Monthly	ISIS 301012	80	Once Monthly	1 to 105	1 to 105
6. 147764	ISIS 147764	60	Once Weekly	1 to 105	1 to 105

^a Dosing ceased when survival reached 20 within a sex in a particular group, with terminal necropsy then occurring when survival subsequently reached 15.

^b Group 4 males were sacrificed at Week 95, Group 3 females at week 98, Group 4 females at week 83.

Dosing was justified as follows: “The dose levels were selected by the Sponsor on the basis of available pharmacology and toxicology data from previous studies . . . The doses of 5, 20, and 60 mg/kg/week were selected on the basis of toxicity, pharmacodynamic, and pharmacokinetic endpoints. The high dose of 60 mg/kg/week was considered to be a maximum tolerated dose (MTD) for a 2-year study. MTD is defined by a combination of significant induction of immune stimulation and hepatocellular changes which were associated with alterations in serum chemistry parameters . . . the top dose of 60 mg/kg/week was expected to produce wide spread proinflammatory effects, without producing effects that would limit life expectancy. The dose level of 80 mg/kg/month was selected as another variation of the 20 mg/kg/week dose level, differing in dosing frequency (monthly administration rather than weekly).

“Doses were also selected on the basis of saturation of renal concentrations and spillage into urine. There was only a 1.2-fold increase in kidney concentration between the doses of 25 and 75 mg/kg/week, and raising the dose above 60 mg/kg/week would artificially increase urinary concentrations of the drug. Studies with related phosphorothioate oligonucleotides have also demonstrated that doses higher than 60 mg/kg/week resulted in marked increases in urinary excretion that are secondary to saturation of plasma protein binding⁴. Doses higher than the proposed dose of 60 mg/kg/week were predicted to produce little increase in exposure but increased urinary excretion that is not representative of a therapeutically active dose range.

“The dose of 60 mg/kg/week ISIS 147764 was based on the dose required to inhibit apoB mRNA levels in lean mice of normal chow diet. In the 6-month mouse toxicology study, a dose of 44 mg/kg/week produced a 30 to 40% inhibition of apoB mRNA level.” (pages 20-21 of mice report).

After randomization, animals were housed individually with food and water available *ad libitum*.

3.2.1.1. Sponsor’s Results and Conclusions

This section will present a summary of the Sponsor’s analysis on survivability and tumorigenicity in mice.

Survival Analysis:

The Sponsor summarizes results as follows: “The number of study animals surviving to the scheduled terminal necropsy (necropsy count)

Table 22. Sponsor Unnumbered Text (copied from page 31)

The number of animals surviving to the scheduled terminal necropsy (Week 105) ^a		
Dose Level	Male	Female
0	24 (34%)	24 (34%)
5 mg/kg/week ISIS 301012	25 (37%)	20 (33%)
20 mg/kg/week ISIS 301012	22 (31%)	0 (0%)
60 mg/kg/week ISIS 301012	0 (0%)	0 (0%)
80 mg/kg/month ISIS 301012	24 (34%)	19 (29%)
60 mg/kg/week ISIS 147764	22 (33%)	28 (43%)

^aRespective survival percentage calculations include/reflect either death or necropsy at Week 105 of the study for groups surviving to scheduled terminal necropsy . . .

“Thus, the incidence of mortality was generally higher than controls for males and females at 60 mg/kg/week ISIS 301012, as well as females at 20 mg/kg/week ISIS 301012. This was considered a treatment-related effect. In fact, the animals in these respective groups were necropsied early (as requested by the U.S. FDA), with dosing terminating for a sex when survival reached 20, and the animals necropsied when survival reached 15. Therefore, males and females at 60 mg/kg/week ISIS 301012 underwent 95 and 83 weeks of dosing, respectively, and were necropsied on Days 672 and 614, respectively, while females at 20 mg/kg/week underwent 98 weeks of dosing and were necropsied on Day 717.” (page 31 of mice report)

Tumorigenicity analysis:

The Sponsor’s pathology report summarizes tumor analysis results as follows:

“Neoplastic findings - Males

“There were no statistically significant increases in any type of neoplasm in the male mice with either the Fisher Exact test for pair-wise comparisons, the Cochran-Armitage Trend test, the Peto test, the Onset rate test, or the Poly-3 Trend test. With the Poly-3 pair-wise comparisons liver hepatocellular carcinoma was statistically significantly increased in the 20 mg/kg/week ISIS

301012 group (8/70 or 11.43% overall rate, 16.27% adjusted compared to 1/70 or 1.43% overall rate, 2.04% adjusted in the control group). The (b) (4) historical control data for this tumor type in males has a range of 0-10% with a mean incidence of 3.8% (common tumor). This statistically significant increase in hepatocellular carcinomas was considered incidental and not test article-related as it was not dose related and the increase was only slightly greater than the (b) (4) historical control range, while the concurrent control was on the low side of the historical control range.

“Also with the Poly-3 pair-wise comparisons the incidence of malignant lymphoma was statistically significantly increased at 80 mg/kg/month (9/70 or 12.86% overall rate, 18.50% adjusted compared to 1/70 or 1.43% overall rate, 2.00% adjusted in the control group). The (b) (4) historical control data for this tumor type in males has a range of 0-13.3% with a mean incidence of 6.2% (common tumor). This statistically significant increase in malignant lymphoma was considered incidental and not test article-related as the overall rate was within the (b) (4) historical control range, the concurrent control was on the low side of the historical control range, there was no trend for increased malignant lymphomas in this sex, and there was not a similar effect in the females.

“Finally, again only with the Poly-3 pair-wise comparisons, the incidence of fibrosarcoma in the subcutaneous tissue of the skin was statistically significantly increased at 60 mg/kg/week with both ISIS 301012 (4/70 or 5.71% overall rate, 11.05% adjusted for 301012 and 3/70 or 4.29% overall rate, 5.99% adjusted for 147764 compared to 0/70 or 0.00% overall rate and adjusted in the control group). The (b) (4) historical control data for this tumor type in males has a range of 0-3.3% with a mean incidence of 0.4% (rare tumor). This statistically significant increase in fibrosarcomas of the subcutaneous tissue was considered incidental and not test article-related as it was only slightly increased as compared to the (b) (4) historical control, the concurrent control was on the low side of the historical control range, there was no trend for increased fibrosarcomas of the subcutaneous tissue in this sex, and there was not a similar effect in the females. Indeed the incidence at 60 mg/kg/week ISIS 301012 exceeds the (b) (4) historical incidence data in males by a raw incidence of only 2 tumors.

“Neoplastic findings – Females

“In female mice the incidence of benign hepatocellular adenoma was statistically significantly increased in the 60 mg/kg/week ISIS 147764 group by the Cochran-Armitage trend test, the Peto test, the Poly-3 trend test, the Fisher Exact and the Poly-3 pair-wise comparisons (23/69 or 33.33% overall rate, 41.51% adjusted compared to 4/70 or 5.71% overall rate, 9.38% adjusted in the control group). Additionally, the incidence of benign hepatocellular adenoma was statistically significantly increased in the 60 mg/kg/week ISIS 301012 group (11/67 or 16.42% overall rate, 34.56% adjusted rate) by the Poly-3 pair-wise comparison only. The (b) (4) historical control data for this tumor type in females has a range of 0-5.7% with a mean incidence of 1.4% (common tumor). The test article-relatedness of this tumor type in the 60 mg/kg/week ISIS 301012 female group is doubtful since it was statistically significant in only a single comparison of several statistical tests performed, even though it exceeds the (b) (4) historical control.

“The incidence of multicentric hemangiosarcoma was statistically significantly increased at 60 mg/kg/week ISIS 301012, also only by the Poly-3 pair-wise comparisons, (11/70 or 15.71% overall rate, 32.79% adjusted compared to 2/70 or 2.86% overall rate, 4.67% adjusted in the control group). The (b) (4) historical control data for this tumor type in females has a range of 0-15.0% with a mean incidence of 4.6% (common tumor). This statistically significant increase in multicentric hemangiosarcoma was considered incidental and not test article-related as it was essentially within the (b) (4) historical control range while the concurrent control was on the low side of the historical control range.

“No other tumor type was statistically significant in either sex by any of the statistical tests used.” (pages 1363-1365 of mice report)

3.2.1.2. FDA Reviewer's Results

This section will present the Agency findings on survival and tumorigenicity in male and female rats.

Survival analysis:

The following tables (Table 23 for male mice, Table 24 for females) summarize the mortality results for the study groups. The data were grouped for the specified time period, and present the number of deaths during the time interval over the number at risk at the beginning of the interval. The percentage cited is the percent that survived at the end of the interval. In these tables the terminal period only includes those animals were sacrificed. Animals that died of other causes during the terminal period are included in the preceding, but overlapping time period. The Kaplan-Meier survival plots in Appendix 1 provide a more detailed picture of the profile of mortality losses.

Table 23. Summary of Male Mice Survival (dose/weeks dosing)

Period (Weeks)	Vehicle 0/ 1-105	Low 5/1- 105	Medium 20/1- 105	High 25/ 1- 95	Monthly 80/ 1-105	ISIS 147764 10/ 1-105
1-52	5/70 ¹ 93% ²	5/70 93%	4/70 94%	10/70 86%	6/70 91%	5/70 93%
53-78	15/65 71%	14/65 73%	15/66 73%	19/60 59%	14/64 71%	8/65 81%
79-91	8/50 60%	7/51 63%	15/51 51%	14/41 39%	12/50 54%	16/57 59%
92-104	18/42 34%	19/44 36%	14/36 31%	12/27 21%	14/38 34%	19/41 31%
Terminal ³	24	25	22	15	24	22

¹ number of deaths / number at risk

² overall per cent survival to end of period.

³ number of animals that survived to terminal sacrifice.

Table 24 Summary of Female Mice Survival (dose/weeks dosing)

Period (Weeks)	Vehicle 0/ 1-105	Low 5/1- 105	Medium 20/1- 98	High 25/ 1- 83	Monthly 80/ 1-105	ISIS 147764 10/ 1-105
1-52	11/70 ¹ 84% ²	7/70 90%	6/70 91%	7/70 90%	3/70 96%	3/70 96%
53-78	17/59 60%	20/63 61%	17/64 67%	29/63 49%	16/67 73%	11/67 80%
79-91	6/42 51%	9/43 49%	18/47 41%	19/34 21%	18/51 47%	6/56 71%
92-104	12/36 34%	14/34 29%	14/29 21%	0	14/33 27%	22/50 40%
Terminal ³	24	20	15	15	19	28

¹ number of deaths / number at risk

² overall per cent survival to end of period.

³ number of animals that survived to terminal sacrifice.

The following table, Table 25 (a repeat of Table 4 and Table A.1.2 in Appendix 1), summarizes the results from tests comparing survival profiles across study groups in the tumorigenicity data sets:

Table 25. Statistical Significances of Tests of Homogeneity and Trend in Survival in the Mouse Study

Hypothesis Tested	Males		Females	
	Log rank	Wilcoxon	Log rank	Wilcoxon
Mouse Homogeneity over Groups 1-6	0.0012	0.0049	< 0.0001	< 0.0001
Homogeneity over Groups 1-4	0.0004	0.0020	0.0004	0.0026
No trend over Groups 1-4	< 0.0001	0.0003	< 0.0001	0.0044
Homogeneity over Groups 1-3	0.6839	0.6495	0.3498	0.8726
No trend over Groups 1-3	0.4149	0.3829	0.1481	0.6430
No Difference Between Groups 1 vs 3	0.4941	0.4642	0.2108	0.7133
No Difference Between Groups 1 vs 4	0.0084	0.0020	0.0013	0.0520
No Difference Between Groups 1 vs 5	0.7180	0.5746	0.8516	0.5058
No Difference Between Groups 1 vs 6	0.7271	0.8703	0.1329	0.0244
No Difference Between Groups 3 vs 5	0.7793	0.8631	0.1593	0.1592

Figures A.1.3 through A.1.4, in Appendix 1, display survival curves for each mouse gender. From Figure A.1.3, in male mice, the High dose group, Group 4, has the lowest survival, with the other dose groups largely intertwined. These differences are sufficient to result in statistically significant tests of overall homogeneity over all six dose groups, i.e., Groups 1-6, (Logrank $p = 0.0012$, Wilcoxon $p = 0.0049$). The low survival in the High dose groups was sufficient to result in a statistically significant test of homogeneity over the more comparable Vehicle to High dose groups, Groups 1-4, (Logrank $p = 0.0004$, Wilcoxon $p = 0.0020$) as well as a statistically significant test of trend over these four groups (Logrank $p < 0.0001$, Wilcoxon $p = 0.0003$). Pairwise comparisons between the vehicle and the High dose group were statistically significant (Logrank $p = 0.0084$, Wilcoxon $p = 0.0020$). None of the other six tests above were

statistically significant (all Logrank $p \geq 0.4149$, Wilcoxon $p \geq 0.3829$). Note that the comparison between groups 3 and 5 involves groups with roughly equal total monthly doses (assuming constant weight), but with group 3 (i.e., the Medium dose group) dosed in four weeks and group 5 (i.e. the Monthly dose group) dosed in one monthly bolus.

From Figure A.1.4, in female mice, by the end of the study the High dose group has the lowest survival, though at first the Vehicle group has the lowest survival. At the end of the study, the ISIS 147764 dose group has the highest survival, with the other dose groups largely intertwined. These differences are sufficient to result in statistically significant tests of overall homogeneity over all six dose groups, Groups 1-6 (both Logrank and Wilcoxon $p < 0.0001$), as well as the test of trend over the four comparable groups, Groups 1-4, (Logrank $p < 0.0001$, Wilcoxon $p = 0.0044$). Pairwise comparisons between the vehicle and the high dose groups were statistically significant or close to significance (Logrank $p = 0.0013$, Wilcoxon $p = 0.0520$), while the comparison to ISIS 147764 was equivocal (Logrank $p = 0.1329$, Wilcoxon $p = 0.0244$). But recall that the log rank test emphasizes later differences, while the Wilcoxon test is more sensitive to earlier differences. None of the other six tests above were statistically significant (Logrank $p \geq 0.1593$, Wilcoxon $p \geq 0.1592$).

In particular, note that for both mouse genders there is no evidence of differences in survival between the 20mg/kg weekly dose and the 80 mg/kg monthly dose of ISIS 301012 (i.e., Males: Logrank $p = 0.7793$, Wilcoxon $p = 0.08631$, Females: Logrank $p = 0.1593$, Wilcoxon $p = 0.1592$).

Tumorigenicity analysis:

Those organ-tumor combinations with at least one nominally statistically significant result ($p \leq 0.05$) in mice are summarized below:

Table 26. Potentially Statistically Significant Neoplasms in Mice

gender organ tumor	Incidence					Significance levels				
	PBS	Low	Med	Hi	Mnth	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1/ 5vs3	2vs1/ 6vs1
Male Mice										
Systemic										
# Evaluated	70	70	70	70	70	70				
Adjusted # at risk	50.4	51.3	48.2	37.4	49.0	49.4				
LYMPHOMA	1	5	3	3	9	0	.2800	.2042	.2933	.1069
							.3436	.0065	.0601	1
liver										
# Evaluated	70	70	70	70	70	70				
Adjusted # at risk	49.5	49.8	49.5	36.0	46.4	51.0				
CARCINOMA, HEPATO- CELLULAR	1	2	8	2	1	4	.2512	.3747	.0153	.5000
							.0038	.7366	.9983	.1938

Table 26. (cont.) Potentially Statistically Significant Neoplasms in Mice

gender organ tumor	Incidence					Mnth	Significance levels			
	PBS	Low	Med	Hi	I14		trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1/ 5vs3	2vs1/ 6vs1
Male Mice (cont.)										
skin, subcutis										
# Evaluated	70	70	70	70	70	70				
Adjusted # at risk	49.4	49.7	47.2	36.9	46.5	50.5				
FIBROSARCOMA	0	0	1	4	0	3	.0021	.0291	.4896	.
							.3241	.	1	.1250
Adjusted # at risk	50.4	49.7	47.2	37.2	46.5	50.5				
Sarcoma/Fibro-/Lipo-	2	0	1	5	0	3	.0072	.1130	.8671	1
							.6913	1	1	.5000
testes										
# Evaluated	70	69	70	70	70	70				
Adjusted # at risk	49.4	48.8	47.7	35.0	46.6	49.4				
ADENOMA, INTERSTITIAL CELL	0	2	4	0	2	1	.6464	.	.0537	.2423
							.0324	.2318	.8933	.5000
Female Mice										
Systemic										
# Evaluated	70	70	70	70	70	70				
Adjusted # at risk	43.1	44.6	42.4	33.9	49.9	53.2				
HEMANGIOSARCOMA	2	8	6	11	9	2	.0025	.0012	.1250	.0482
							.2073	.0416	.4075	.7660
Adjusted # at risk	43.9	44.6	42.7	34.6	49.9	53.2				
Hemangioma/-sarcoma	3	8	7	12	9	4	.0024	.0022	.1471	.1050
							.1879	.0940	.5272	.6163
harderian glands										
# Evaluated	70	70	68	70	70	70				
Adjusted # at risk	42.3	43.6	41.0	29.9	46.8	54.4				
ADENOMA	1	3	3	4	4	3	.0547	.0857	.2988	.3169
							.2360	.2099	.5649	.4088
liver										
# Evaluated	70	70	68	67	70	69				
Adjusted # at risk	42.9	43.0	41.9	32.2	47.3	55.7				
ADENOMA, HEPATOCELLULAR	4	0	5	11	5	23	<0.0001	.0095	.4844	1
							.1623	.5722	.7148	.0003
Adjusted # at risk	42.3	43.0	40.1	27.5	46.2	53.1				
CARCINOMA, HEPATO- CELLULAR	0	0	0	2	0	2	.0306	.1496	.	.
						3086
Adjusted # at risk	42.9	43.0	41.9	32.6	47.3	56.0				
Hepato. Adenoma/-carc.	4	0	5	12	5	25	<0.0001	.0044	.4844	1
							.1623	.5722	.7148	.0001
lung										
# Evaluated	70	70	70	70	70	70				
Adjusted # at risk	44.3	45.6	45.6	33.7	49.4	54.2				
ADENOMA, BRONCH. ALV.	10	9	17	11	9	8	.0955	.2185	.0942	.7163
							.0346	.7818	.9905	.8974

In male mice, the tests of trend over Groups 1-4 in fibrosarcoma of the subcutis was statistically significant ($p = 0.0021 < 0.025$) as was the pairwise comparison between the high dose and control ($p = 0.0291 < 0.05$). However the test of trend in pooled sarcoma, fibrosarcoma, and liposarcoma would not be quite statistically significant ($p = 0.0072 > 0.005$). Although the test of trend over the four 301012 groups in hepatocellular carcinoma of the liver would not be classified as statistically significant, accepting the inflation of overall type I error, the corresponding test of trend over the first three groups would be ($p = 0.0038 < 0.005$).

Differences between the monthly dose and vehicle in systemic lymphoma could be attributed to differences in drug or dosing schedule, but accepting the inflation in Type I error, it would be classified as statistically significant ($p = 0.0065 < 0.01$). No other tests or comparisons achieved the Haseman-Lin-Rahman multiplicity adjusted levels of significance, although the test of trend over the first three doses and difference between the medium dose and vehicle in interstitial cell adenoma of the testes would both be close ($p = 0.0324 > 0.025$, $p = 0.0537 \approx 0.05$, respectively).

In female mice, the tests of trend over Groups 1-4 in systemic hemangiosarcoma and pooled hemangioma and hemangiosarcoma were both statistically significant ($p=0.0025$, $0.0024 < 0.005$), as were the similar tests of trend in hepatocellular adenoma and pooled hepatocellular adenoma and carcinoma of the liver (both $p < 0.0001 < 0.005$). Pairwise comparisons between the high dose and PBS vehicle in systemic hemangiosarcoma and pooled hemangioma and hemangiosarcoma were also both statistically significant ($p=0.0012$, $0.0022 < 0.01$), as was the similar comparison in hepatocellular adenoma and pooled hepatocellular adenoma and carcinoma of the liver ($p = 0.0095$, $0.0044 < 0.01$). The comparisons between ISIS 147764 and PBS vehicle in hepatocellular adenoma and pooled hepatocellular adenoma and carcinoma of the liver would also be classified as statistically significant ($p = 0.0003$, $0.0001 < 0.01$). Again, no other comparisons met the multiplicity/rarity adjusted test significance levels.

Tediously complete incidence tables in both genders are provided in tables A.2.5 and A.2.6 in Appendix 3.

4. FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

NA

5. SUMMARY AND CONCLUSIONS

5.1. Statistical Issues and Collective Evidence

Please see Section 1.3 above.

5.2. Conclusions and Recommendations

Please see Section 1.1 above.

APPENDICES

Appendix 1. FDA Survival Analysis

Simple summary life tables in mortality are presented in the report (Tables 14, 15, 19, and 20 above). Kaplan-Meier estimated survival curves across study groups for each gender are displayed below in Figures A.1.1 and A.1.2 for rats and Figures A.1.3 and A.1.4 for mice. These plots include 95% confidence intervals around each survival curve (colored area around each curve). These plots are also supported by tests of homogeneity in survival over the five (in rats) and six (in mice) different treatment groups, tests of homogeneity and trend in comparable groups differing only in dose, and the results of pairwise comparisons to the vehicle. The statistical significance levels (i.e., p-values) are provided in Tables A.1.1. and A.1.2., below. One might note that the log rank tests places greater weight on later events, while the Wilcoxon test tends to weight them more equally, and thus places more weight on differences in earlier events than does the log rank test.

Table A.1.1. Statistical Significances of Tests of Homogeneity and Trend in Survival in the Rat Study

Hypothesis Tested	Males		Females	
	Log rank	Wilcoxon	Log rank	Wilcoxon
Rat Homogeneity over Groups 1-5	< 0.0001	< 0.0001	0.0022	0.0114
Homogeneity over Groups 1-4	< 0.0001	< 0.0001	0.0006	0.0060
No trend over Groups 1-4	< 0.0001	< 0.0001	0.0003	0.0023
Homogeneity over Groups 1-3	0.0001	0.0005	0.0489	0.2197
No trend over Groups 1-3	< 0.0001	0.0002	0.1702	0.4727
No Difference Between Groups 1 vs 3	< 0.0001	< 0.0001	0.0802	0.2924
No Difference Between Groups 1 vs 4	< 0.0001	< 0.0001	0.0023	0.0046
No Difference Between Groups 1 vs 5	0.6482	0.6580	0.2447	0.3097

From Figure A.1.1, in male rats, at first there is a clear decreasing survival over dose with exception that Group 5, the ISIS 147768 group, closely tracks the group with the highest survival, the vehicle group, Group 1. This is consistent with the results of the tests above. With the exception of the pairwise comparison between the vehicle and the ISIS 147768 group (Log rank $p = 0.6482$, Wilcoxon $p = 0.6580$), all tests of trend, homogeneity, and pairwise comparisons are highly statistically significant (all seven Logrank $p \leq 0.0001$, all Wilcoxon $p \leq 0.0005$).

Results in female rats are not quite as clear or simple as in the male rats above. From Figure A.1.2, the high dose, Group 4, has the lowest survival, particularly later in the study. The vehicle group, Group 1, has the highest or close to highest survival. The other groups were more or less intertwined between these two groups. These differences are sufficient to result in statistically significant tests of overall homogeneity over all five dose groups, Groups 1-5 (Logrank $p = 0.0022$, Wilcoxon $p = 0.0114$), and homogeneity over the Vehicle to High dose, Groups 1-4 (Logrank $p = 0.0006$, Wilcoxon $p = 0.0060$). Tests of homogeneity over the Vehicle

to Medium dose groups, Groups 1-3, were much more equivocal (Logrank $p = 0.0489$, Wilcoxon $p = 0.2924$), with weak evidence of later differences among these groups. The lower survival in the High dose group is sufficient to result in a statistically significant in Groups 1-4 (Logrank $p = 0.0003$, Wilcoxon $p = 0.0023$), but there is no evidence of a dose related trend in Groups 1-3 (Logrank $p = 0.1702$, Wilcoxon $p = 0.4727$). Pairwise comparisons between the vehicle and the high dose groups were statistically significant (Logrank $p = 0.0023$, Wilcoxon $p = 0.0046$), but pairwise comparisons to the medium and ISIS 147768 dose groups were not statistically significant (Logrank $p = 0.0802, 0.2447$, Wilcoxon $p = 0.2924, 0.3097$ respectively).

Figure A.1.1 Kaplan-Meier Survival Curves for Male Rats

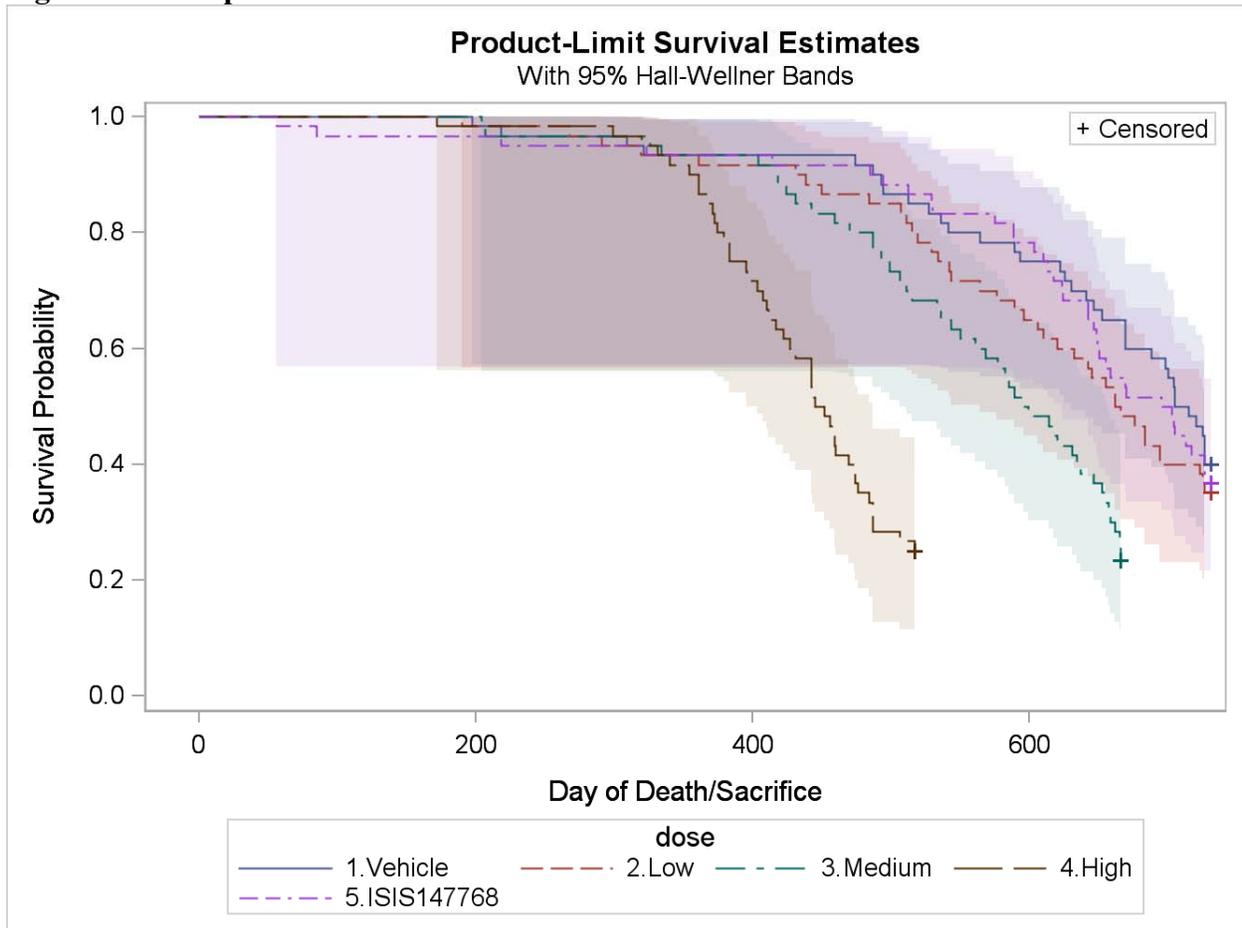
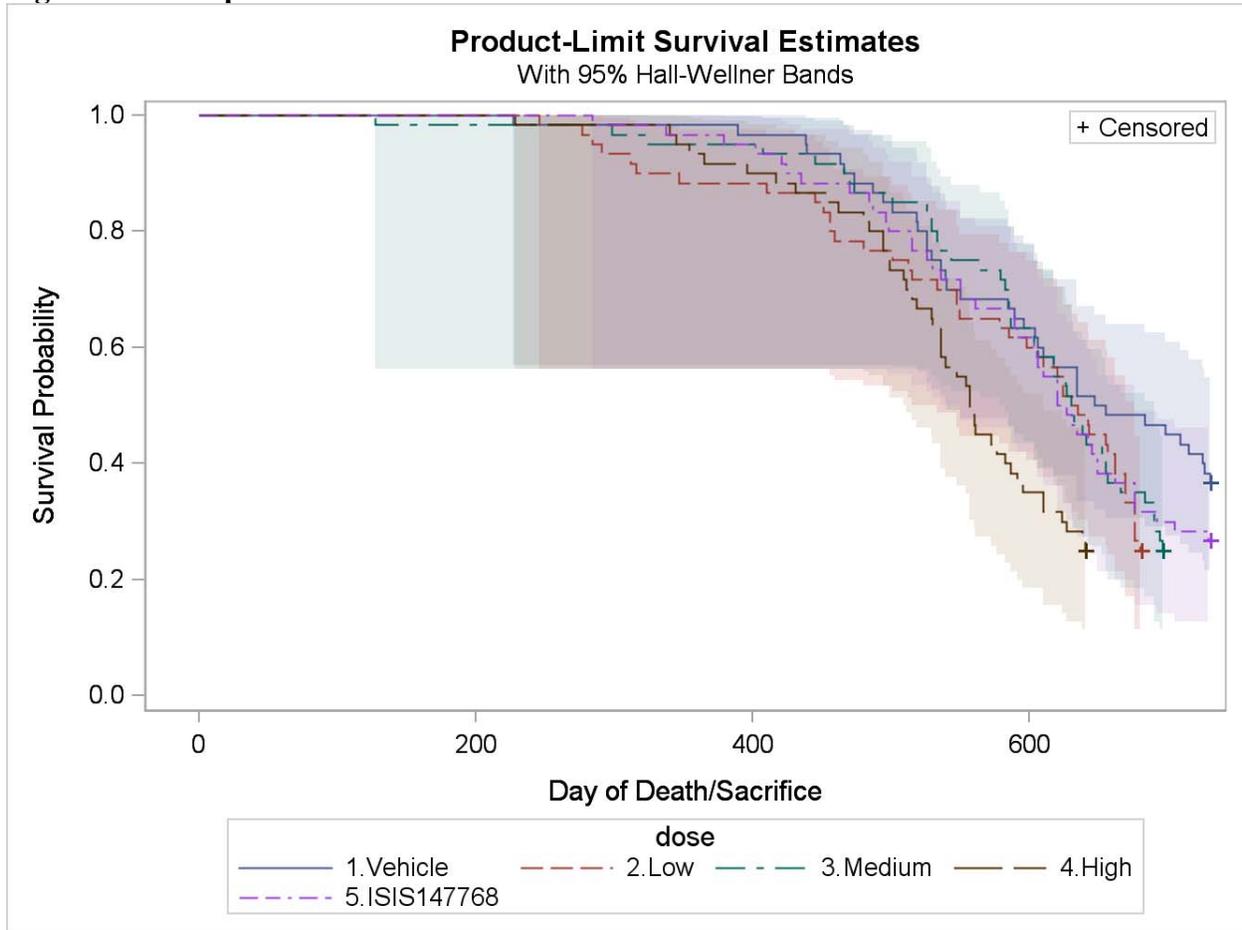


Figure A.1.2 Kaplan-Meier Survival Curves for Female Rats



Results for similar tests in mice are presented below:

Table 4. Statistical Significances of Tests of Homogeneity and Trend in Survival in the Mouse Study

Hypothesis Tested	Males		Females	
	Log rank	Wilcoxon	Log rank	Wilcoxon
Mouse Homogeneity over Groups 1-6	0.0012	0.0049	< 0.0001	< 0.0001
Homogeneity over Groups 1-4	0.0004	0.0020	0.0004	0.0026
No trend over Groups 1-4	< 0.0001	0.0003	< 0.0001	0.0044
Homogeneity over Groups 1-3	0.6839	0.6495	0.3498	0.8726
No trend over Groups 1-3	0.4149	0.3829	0.1481	0.6430
No Difference Between Groups 1 vs 3	0.4941	0.4642	0.2108	0.7133
No Difference Between Groups 1 vs 4	0.0084	0.0020	0.0013	0.0520
No Difference Between Groups 1 vs 5	0.7180	0.5746	0.8516	0.5058
No Difference Between Groups 1 vs 6	0.7271	0.8703	0.1329	0.0244
No Difference Between Groups 3 vs 5	0.7793	0.8631	0.1593	0.1592

From Figure A.1.3, below, in male mice, the High dose group, Group 4, has the lower survival, with the other dose groups largely intertwined. These differences are sufficient to result in statistically significant tests of overall homogeneity over all six dose groups, Groups 1-6 (Logrank $p = 0.0012$, Wilcoxon $p = 0.0049$). The low survival in the High dose groups was sufficient to result in a statistically significant test of homogeneity over the more comparable Vehicle to High dose groups, Groups 1-4, (Logrank $p = 0.0004$, Wilcoxon $p = 0.0020$) as well as a statistically significant test of trend over these four groups (Logrank $p < 0.0001$, Wilcoxon $p = 0.0003$). Pairwise comparisons between the vehicle and the high dose groups were statistically significant (Logrank $p = 0.0084$, Wilcoxon $p = 0.0020$). None of the other six tests and comparisons above were statistically significant (Logrank $p \geq 0.4149$, Wilcoxon $p \geq 0.3829$).

From Figure A.1.4, in female mice, by the end of the study the High dose group has the lowest survival, though at first the Vehicle group has the lowest survival. At the end of the study, the ISIS 147764 dose group has the highest survival, with the other dose groups largely intertwined. These differences are sufficient to result in statistically significant tests of overall homogeneity over all six dose groups, Groups 1-6 (both Logrank and Wilcoxon $p < 0.0001$), as well as the test of trend over the four comparable groups, Groups 1-4, (Logrank $p < 0.0001$, Wilcoxon $p = 0.0044$). As with male mice, pairwise comparisons between the vehicle and the high dose groups were statistically significant (Logrank $p = 0.0013$, Wilcoxon $p = 0.0520$), while the comparison to ISIS 147764 was equivocal (Logrank $p = 0.1329$, Wilcoxon $p = 0.0244$). None of the other six tests above were statistically significant at the usual 0.05 level (Logrank $p \geq 0.1481$, Wilcoxon $p \geq 0.1592$).

In particular note that there is no evidence of differences in survival between the 20 mg/kg weekly dose and the 80 mg/kg monthly dose of ISIS 301012 (i.e., Males: Logrank $p = 0.7793$, Wilcoxon $p = 0.08631$, Females: Logrank $p = 1593$, Wilcoxon $p = 0.1592$).

Figure A.1.3 Kaplan-Meier Survival Curves for Male Mice

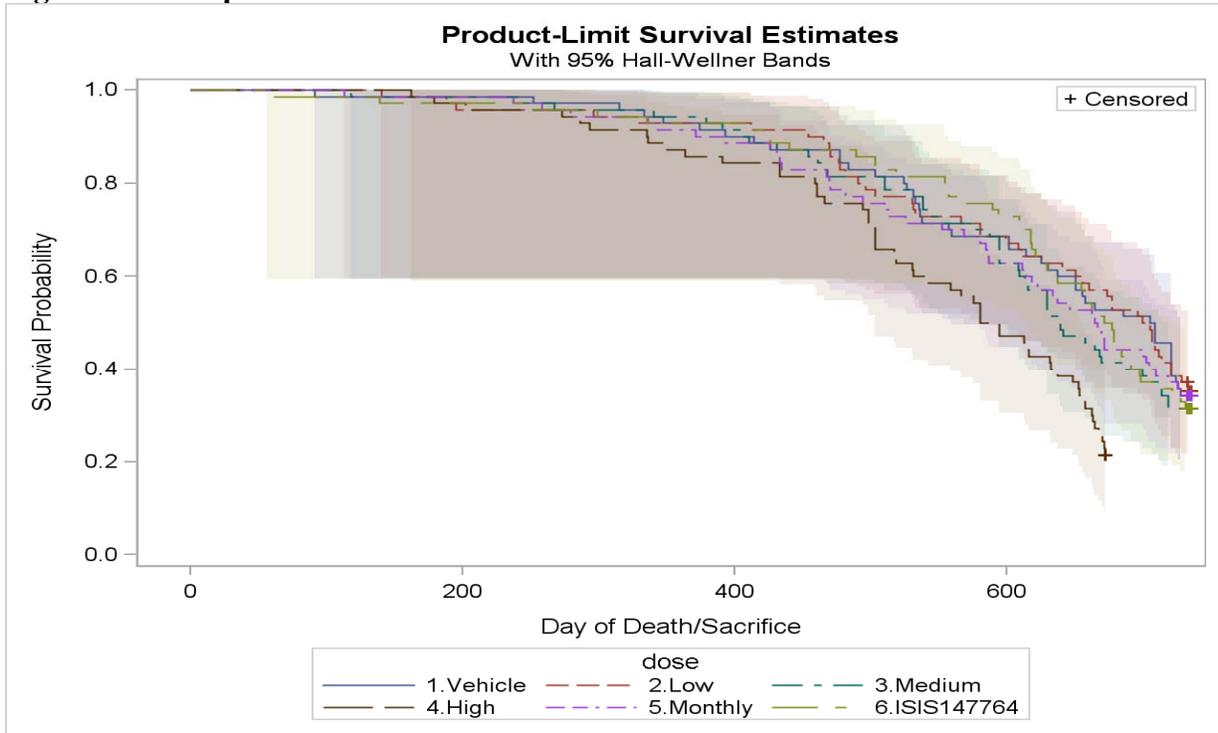
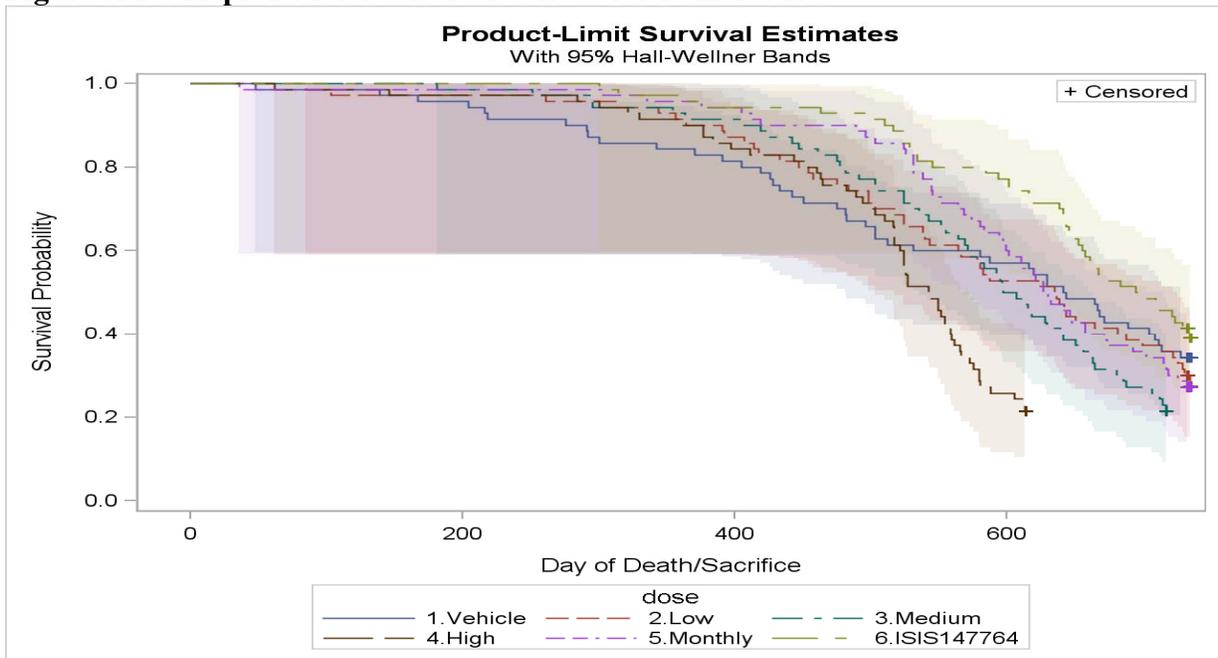


Figure A.1.4 Kaplan-Meier Survival Curves for Female Mice



Appendix 2. FDA Poly-k Tumorigenicity Analysis

The poly-k test, here with $k=3$, modifies the original Cochran-Armitage test to adjust for differences in mortality (please see Bailer & Portier, 1988, Bieler & Williams, 1993). The tests used here are small sample exact permutation tests of tumor incidence. When there were no tumors of the specific type being analyzed in either column of the 2x2 table corresponding to a pairwise comparison an argument could be made that the p-value for this test should be 1.0. However, largely for readability, in the tables below these p-values are considered as missing (i.e., corresponding to a null test), denoted by a period “.”. Note that the StatXact program used for these analyses adjusts for the variance, which would be 0. Then the significance levels of the test statistics are based on the result of a division by 0, i.e., undefined, and hence StatXact codes these p-values as missing.

For each species by gender by organ the number of animals analyzed and used in the statistical tests is presented first. Note that indicating an organ was not examined requires a specification in the data (please see section 2.2 above). For those tables with statistically significant tests or comparisons (i.e., $p\text{-value} \leq 0.05$), the adjusted number of animals at risk is presented next. As discussed in Section 1.3.1.3, above, this is the sum of the poly-k weights for each tumor within each organ, and represents the number of animals at risk of that tumor by downweighting those animals that die early without the tumor. Note this adjusted number of animals at risk applies to the tumor immediately this entry. For all tables the tumor incidence is presented next, with the significance levels of the tests of trend, and the results of pairwise tests between the high, medium, and low dose groups and vehicle, plus Incidence in the vehicle group is used to assess background tumor incidence, and thus whether a tumor is considered to be rare (background incidence $<1\%$) or common.

To adjust for the multiplicity of tests the so-called Haseman-Lin-Rahman (HLR) rules discussed in Section 1.3.1.4 are often applied. That is, when testing for trend over dose groups 1-4 and the difference between the highest dose group with a control group, to control the overall Type I error rate to roughly 10% for a standard two species, two sex study, one compares the unadjusted significance level of the trend test to 0.005 for common tumors and 0.025 for rare tumors, and the pairwise test to 0.01 for common tumors and 0.05 for rare tumors. Using these adjustments for other tests, like testing trend over groups 1-3, and the comparisons between the low, medium dose groups and the ISIS 147768 group with vehicle, and, in mice, the comparisons with the ISIS 301012 low and medium weekly and monthly dose groups, and the ISIS 147764 dose group can be expected to increase the overall type I error rate to some value above the nominal rough 10% level, possibly considerably higher than the nominal 10% rate.

Table A.2.1 in rats and Table A.2.2 in mice show the tumors that had at least one mortality adjusted test whose nominal statistical significance was at least no more than 0.05. Note that when one adjusts for multiplicity these nominally significant comparisons may not be statistically significant. Tables A.2.3 and A.2.4 display all incidences and statistical test results for male and female rats, respectively, while Tables A.2.5 and A.2.6 present similar results in male and female mice. The p-values of the poly-k test are based on exact tests from StatXact as

discussed above. As also noted above, the period ‘.’ denotes the p-values of tests of dose groups with no tumors in any group. Note that an animal that dies early without a tumor reduces the size of the risk set for that tumor. The poly-k test down weights such animals and a better estimate of the number at risk is given in the row labeled “Adjusted # at risk”. Recall that “I14” denotes the ISIS 147768 dose group in rats and the ISIS 147764 dose group in mice.

Table A.2.1 Potentially Statistically Significant Neoplasms in Rats

Gender/ organ tumor	Incidence					Significance levels			
	PBS	Low	Med	Hi	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1	2vs1
Male Rats									
pituitary gland									
# Evaluated	60	60	60	60	60				
Adjusted # at risk	51.3	54.5	43.5	23.5	51.3				
ADENOMA, PARS DISTALIS	36	47	29	13	36	.9782 .7910	.9253 .5859	.7103	.0331
skin, subcutis									
# Evaluated	60	60	60	60	60				
Adjusted # at risk	46.0	42.4	32.3	16.4	44.9				
FIBROUS HISTIOCYTOMA	0	1	3	3	1	.0046 .0301	.0148 .4889	.0652	.4713
Adjusted # at risk	46.1	41.5	32.7	17.1	46.4				
Fibroma/Fibrosarcoma/Fibrous Histiocytoma	1	6	5	4	4	.0245 .0490	.0165 .1805	.0396	.0423
Female Rats									
Uterus/Cervix/Vagina									
# Evaluated	60	60	60	60	60				
Adjusted # at risk	42.2	34.2	38.9	31.1	38.8				
GRANULAR CELL TUMOR	4	2	6	10	4	.0016 .1537	.0164 .5858	.3057	.8437
ovaries									
# Evaluated	60	60	60	60	60				
Adjusted # at risk	41.8	33.8	37.1	28.6	38.6				
GRANULOSA CELL TUMOR	0	0	0	2	1	.0394 .	.1611 .4810	.	.
pituitary gland									
# Evaluated	60	60	60	60	60				
Adjusted # at risk	54.9	54.4	52.9	41.1	55.4				
ADENOMA, PARS DISTALIS	43	51	45	27	50	.9964 .2873	.9592 .0812	.2463	.0210
skin, subcutis									
# Evaluated	60	60	60	60	60				
Adjusted # at risk	41.8	34.0	38.1	29.6	38.5				
FIBROSARCOMA	0	1	4	5	1	.0046 .0215	.0098 .4810	.0491	.4533
Adjusted # at risk	42.4	34.2	38.9	30.0	38.5				
Fibroma/Fibrosarcoma	1	2	6	6	1	.0103 .0187	.0183 .7275	.0405	.4203
Adjusted # at risk	41.8	33.8	38.3	29.8	38.2				
FIBROUS HISTIOCYTOMA	0	0	3	4	0	.0053 .0370	.0259 .	.1067	.
Adjusted # at risk	42.4	34.2	40.2	32.4	38.5				
Fibroma/Fibrosarcoma/Fibr. Histiocytoma	1	2	9	10	1	.0003 .0016	.0007 .7275	.0058	.4203

Table A.2.1 (cont.) Potentially Statistically Significant Neoplasms in Rats

Gender/ organ tumor	Incidence					Significance levels			
	PBS	Low	Med	Hi	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1	2vs1
Female Rats (cont.)									
thyroid gland									
# Evaluated	60	60	60	60	60				
Adjusted # at risk	42.5	35.3	41.6	28.5	38.8				
C-Cell Adenoma/Carcinoma	2	5	9	3	7	.3373 .0183	.3122 .0564	.0218	.1474
uterus with cervix									
# Evaluated	60	60	60	60	60				
Adjusted # at risk	41.8	33.9	38.1	30.5	38.2				
GRANULAR CELL TUMOR	2	1	3	8	1	.0008 .2910	.0117 .8652	.4635	.8356

In male rats, the tests of trend in fibrous histiocytoma, of the subcutis, over Groups 1-4 is statistically significant ($p = 0.0046 < 0.025$), and, accepting the increase in Type I error from including this test, the test of trend over Groups 1-3 is not quite statistically significant ($p = 0.0301 > 0.025$), nor is either test of trend in pooled fibroma, fibrosarcoma, and fibrous histiocytoma ($p = 0.0245, 0.0490 > 0.005$, respectively). For this pooled tumor the pairwise comparison between the high dose and the vehicle is also not quite statistically significant ($p = 0.0165 > 0.01$). However, the test of differences between the high dose group and vehicle (i.e., Groups 4 versus 1) in fibrous histiocytoma of the subcutis is statistically significant ($p = 0.0148 < 0.05$). The test comparing the low dose to vehicle (i.e. Groups 2 versus 1) in pars distalis adenoma of the pituitary is not statistically significant ($p = 0.0331 > 0.01$).

In female rats, the tests of trend in granular cell tumor in both the uterus with cervix and adding the vagina, over the four ISIS 301012 treatment groups including vehicle, (i.e. Groups 1-4) were statistically significant ($p = 0.0016, 0.0008 < 0.005$, respectively). Similarly the equivalent tests of trend in these groups in fibrosarcoma and fibrous histiocytoma in the subcutis were also statistically significant ($p = 0.0046, 0.0053 < 0.025$), as was the test of trend for fibrosarcoma over the first three doses ($p = 0.0215 < 0.025$). Further, in the subcutis, the tests of trend in pooled fibroma, fibrosarcoma, and fibrous histiocytoma were statistically significant over both groups 1-4 and 1-3 ($p = 0.0003, 0.0016 < 0.005$, respectively). The test of trend in pooled fibroma and fibrosarcoma was not statistically significant over either groups 1-4 and 1-3 ($p = 0.0103, 0.0187 > 0.005$, respectively). For pairwise tests, again accepting the increase in Type I error, the difference between the high and medium dose groups in fibrosarcoma in the subcutis would be statistically significant ($p = 0.0098, 0.0491 < 0.05$, respectively). In the subcutis the pairwise comparison between the high dose and control in fibrous histiocytoma would also be statistically significant ($p = 0.0259 < 0.05$). The pairwise comparisons between the high and medium dose groups with vehicle in terms pooled fibroma, fibrosarcoma, and fibrous histiocytoma of the subcutis were statistically significant ($p = 0.0007, 0.0058 < 0.01$, respectively). Finally, in the uterus with cervix the comparison between the high dose and control in granular cell tumor was close to statistical significance ($p = 0.0117 \approx 0.01$).

Those organ-tumor combinations with at least one nominally statistically significant result ($p \leq 0.05$) in mice are summarized below:

Table A.2.2 Potentially Statistically Significant Neoplasms in Mice

gender organ tumor	Incidence						Significance levels			
	PBS	Low	Med	Hi	Mnth	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1/ 5vs3	2vs1/ 6vs1
Male Mice										
Systemic										
# Evaluated	70	70	70	70	70	70				
Adjusted # at risk	50.4	51.3	48.2	37.4	49.0	49.4				
LYMPHOMA	1	5	3	3	9	0	.2800 .3436	.2042 .0065	.2933 .0601	.1069 1
liver										
# Evaluated	70	70	70	70	70	70				
Adjusted # at risk	49.5	49.8	49.5	36.0	46.4	51.0				
CARCINOMA, HEPATO- CELLULAR	1	2	8	2	1	4	.2512 .0038	.3747 .7366	.0153 .9983	.5000 .1938
skin, subcutis										
# Evaluated	70	70	70	70	70	70				
Adjusted # at risk	49.4	49.7	47.2	36.9	46.5	50.5				
FIBROSARCOMA	0	0	1	4	0	3	.0021 .3241	.0291 .	.4896 1	. .1250
Adjusted # at risk	50.4	49.7	47.2	37.2	46.5	50.5				
Sarcoma/Fibro-/Lipo-	2	0	1	5	0	3	.0072 .6913	.1130 1	.8671 1	1 .5000
testes										
# Evaluated	70	69	70	70	70	70				
Adjusted # at risk	49.4	48.8	47.7	35.0	46.6	49.4				
ADENOMA, INTERSTITIAL CELL	0	2	4	0	2	1	.6464 .0324	. .2318	.0537 .8933	.2423 .5000
Female Mice										
Systemic										
# Evaluated	70	70	70	70	70	70				
Adjusted # at risk	43.1	44.6	42.4	33.9	49.9	53.2				
HEMANGIOSARCOMA	2	8	6	11	9	2	.0025 .2073	.0012 .0416	.1250 .4075	.0482 .7660
Adjusted # at risk	43.9	44.6	42.7	34.6	49.9	53.2				
Hemangioma/-sarcoma	3	8	7	12	9	4	.0024 .1879	.0022 .0940	.1471 .5272	.1050 .6163
harderian glands										
# Evaluated	70	70	68	70	70	70				
Adjusted # at risk	42.3	43.6	41.0	29.9	46.8	54.4				
ADENOMA	1	3	3	4	4	3	.0547 .2360	.0857 .2099	.2988 .5649	.3169 .4088
liver										
# Evaluated	70	70	68	67	70	69				
Adjusted # at risk	42.9	43.0	41.9	32.2	47.3	55.7				
ADENOMA, HEPATOCELLULAR	4	0	5	11	5	23	<0.0001 .1623	.0095 .5722	.4844 .7148	1 .0003
Adjusted # at risk	42.3	43.0	40.1	27.5	46.2	53.1				
CARCINOMA, HEPATO- CELLULAR	0	0	0	2	0	2	.0306 .	.14963086
Adjusted # at risk	42.9	43.0	41.9	32.6	47.3	56,0				
Hepato. Adenoma/-carc.	4	0	5	12	5	25	<0.0001 .1623	.0044 .5722	.4844 .7148	1 .0001
lung										
# Evaluated	70	70	70	70	70	70				
Adjusted # at risk	44.3	45.6	45.6	33.7	.4	54.2				
ADENOMA, BRONCH. ALV.	10	9	17	11	9	8	.0955 .0346	.2185 .7818	.0942 .9905	.7163 .8974

In male mice, the tests of trend over Groups 1-4 in fibrosarcoma of the subcutis was statistically significant ($p = 0.0021 < 0.025$) as was the pairwise comparison between the high dose and control ($p = 0.0291 < 0.05$). However the test of trend in pooled sarcoma, fibrosarcoma, and liposarcoma would not be quite statistically significant ($p = 0.0072 > 0.005$). Although the test of trend over the four 301012 groups in hepatocellular carcinoma of the liver would not be classified as statistically significant, accepting the inflation of overall type I error, the corresponding test of trend over the first three groups would be ($p = 0.0038 < 0.005$). Differences between the monthly dose and vehicle in systemic lymphoma could be attributed to differences in drug or dosing schedule, but accepting the inflation in Type I error, it would be classified as statistically significant ($p = 0.0065 < 0.01$). No other tests or comparisons achieved the Haseman-Lin-Rahman multiplicity adjusted levels of significance, although the test of trend over the first three doses and difference between the medium dose and vehicle in interstitial cell adenoma of the testes would both be close ($p = 0.0324 > 0.025$, $p = 0.0537 \approx 0.05$, respectively).

In female mice, the tests of trend over Groups 1-4 in systemic hemangiosarcoma and pooled hemangioma and hemangiosarcoma were both statistically significant ($p=0.0025$, $0.0024 < 0.005$), as were the similar tests of trend in hepatocellular adenoma and pooled hepatocellular adenoma and carcinoma of the liver (both $p < 0.0001 < 0.005$). Pairwise comparisons between the high dose and PB vehicle in systemic hemangiosarcoma and pooled hemangioma and hemangiosarcoma were also both statistically significant ($p=0.0012$, $0.0022 < 0.005$), as was the similar comparison in hepatocellular adenoma and pooled hepatocellular adenoma and carcinoma ($p = 0.0095$, $0.0044 < 0.01$). The comparisons between ISIS 147764 and PBS vehicle in hepatocellular adenoma and pooled hepatocellular adenoma and carcinoma of the liver would also be classified as statistically significant ($p = 0.0003$, $0.0001 < 0.01$). Again, no other comparisons met the multiplicity/rarity adjusted test significance levels.

No other tests or comparisons achieved the Haseman-Lin-Rahman multiplicity adjusted levels of significance. Complete incidence tables in each species by gender combination are presented below:

Table A.2.3 Incidence and Significance Levels of all Tests on Neoplasms in Male Rats

organ tumor	Incidence					Significance levels			
	PBS	Low	Med	Hi	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1	2vs1
Bone Marrow, Overall									
# Evaluated	60	60	60	60	60				
LEUKEMIA, LARGE GRANULAR LYMPHOCYTIC	0	0	0	0	1	.	.4889	.	.
SARCOMA, HISTIOCYTIC	0	1	0	0	2	.6541 .6134	.	.	.4773
Injection Site									
# Evaluated	60	60	60	60	60				
SARCOMA, HISTIOCYTIC	0	0	0	0	1	.	.494	.	.

Table A.2.3 (cont.) Incidence and Significance Levels of all Tests on Neoplasms in Male Rats

organ tumor	Incidence					Significance levels			
	PBS	Low	Med	Hi	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1	2vs1
Large Intestine, Overall									
# Evaluated	60	60	60	60	60				
FIBROSARCOMA	0	1	0	0	0	.6515	.	.	.4713
						.6102	.		
LEIOMYOMA	0	1	0	0	0	.6515	.	.	.4713
						.6102	.		
SARCOMA, HISTIOCYTIC	0	0	0	0	1
						.	.4945		
Lymph Node, Overall									
# Evaluated	60	60	59	57	60				
CARCINOMA, TUBULAR CELL	0	0	0	0	1
						.	.4945		
FIBROSARCOMA	0	1	0	0	0	.6462	.	.	.4713
						.6068	.		
FIBROUS HISTIOCYTOMA	0	0	1	0	0	.3359	.	.4026	.
						.2627	.		
SARCOMA, HISTIOCYTIC	0	0	0	0	1
						.	.4945		
SCHWANNOMA	0	1	0	0	0	.6462	.	.	.4713
						.6068	.		
Nose									
# Evaluated	60	60	60	60	60				
PAPILLOMA	1	0	0	0	0	1	1	1	1
						1	1		
SARCOMA, HISTIOCYTIC	0	1	0	0	0	.6541	.	.	.4773
						.6134	.		
Small Intestine, Overall									
# Evaluated	60	60	60	60	60				
ADENOCARCINOMA	0	2	0	0	0	.6620	.	.	.2249
						.5774	.		
Spinal Cord, Overall									
# Evaluated	60	59	60	60	60				
LEUKEMIA, LARGE GRANULAR LYMPHOCYTIC	0	0	0	0	1
						.	.4889		
Systemic									
# Evaluated	60	60	60	60	60				
HEMANGIOMA	0	0	0	0	1
						.	.4889		
HEMANGIOSARCOMA	3	1	0	0	1	.9857	1	1	.9235
						.9775	.9333		
Hemangioma/hemangiosarcoma	3	1	0	0	2	.9857	1	1	.9235
						.9775	.7983		
LYMPHOMA	2	1	1	0	0	.7750	1	.7925	.8617
						.6471	1		
adipose tissue, brown									
# Evaluated	60	60	60	60	60				
HIBERNOMA	1	0	1	1	2	.1573	.4344	.6463	1
						.4581	.5000		

Table A.2.3 (cont.) Incidence and Significance Levels of all Tests on Neoplasms in Male Rats

organ tumor	Incidence					Significance levels			
	PBS	Low	Med	Hi	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1	2vs1
adrenal glands									
# Evaluated	60	60	60	60	60				
ADENOMA, CORTICAL	2	1	0	0	1	.9595	1	1	.8568
						.9431	.8750		
LEUKEMIA, LARGE GRANULAR LYMPHOCYTIC	0	0	0	0	2
						.	.2362		
Pheochromocytoma	10	7	11	3	10	.4284	.7201	.2563	.8080
						.1381	.5779		
SARCOMA, HISTIOCYTIC	0	0	0	0	1
						.	.4945		
bone marrow, femur									
# Evaluated	60	60	60	60	60				
LEUKEMIA, LARGE GRANULAR LYMPHOCYTIC	0	0	0	0	1
						.	.4889		
SARCOMA, HISTIOCYTIC	0	1	0	0	2	.6541	.	.	.4773
						.6134	.2418		
bone marrow, sternum									
# Evaluated	60	60	60	60	60				
LEUKEMIA, LARGE GRANULAR	0	0	0	0	1
						.	.4889		
SARCOMA, HISTIOCYTIC	0	0	0	0	1
						.	.4945		
brain									
# Evaluated	60	60	60	60	60				
ASTROCYTOMA	1	0	0	1	1	.2016	.4153	1	1
						1	.7473		
CARCINOMA, PARS DISTALIS	0	0	0	0	1
						.	.4889		
LEUKEMIA, LARGE GRANULAR LYMPHOCYTIC	0	0	0	0	1
						.	.4889		
SARCOMA, HISTIOCYTIC	0	1	0	0	0	.6541	.	.	.4773
						.6134	.		
cavity, abdominal									
# Evaluated	60	60	60	60	60				
CARCINOMA, TUBULAR CELL	0	0	0	0	1
						.	.4945		
FIBROMA	1	0	0	0	0	1	1	1	1
						1	1		
FIBROSARCOMA	0	1	0	0	0	.6515	.	.	.4713
						.6102	.		
LIPOSARCOMA	0	0	0	0	1
						.	.4945		
SARCOMA, HISTIOCYTIC	0	0	0	0	1
						.	.4945		
SCHWANNOMA	0	0	0	0	1
						.	.4945		
cavity, thoracic									
# Evaluated	60	60	60	60	60				
FIBROSARCOMA	0	1	0	0	0	.6515	.	.	.4713
						.6102	.		
SARCOMA, HISTIOCYTIC	0	0	0	0	1
						.	.4945		

Table A.2.3 (cont.) Incidence and Significance Levels of all Tests on Neoplasms in Male Rats

organ tumor	Incidence					Significance levels			
	PBS	Low	Med	Hi	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1	2vs1
eyes									
# Evaluated	60	60	60	60	60				
ADENOCARCINOMA	0	0	0	0	1
							.4945		
harderian glands									
# Evaluated	60	60	60	60	60				
ADENOCARCINOMA	0	0	0	0	1
							.4945		
LEUKEMIA, LARGE GRANULAR LYMPHOCYTIC	0	0	0	0	1
							.4889		
heart									
# Evaluated	60	60	60	60	60				
CARCINOMA, TUBULAR CELL	0	0	0	0	1
							.4945		
CARCINOMA, UNDIFFERENTIATED	0	0	0	1	0	.1128	.2459	.	.
							.		
LEUKEMIA, LARGE GRANULAR LYMPHOCYTIC	0	0	0	0	1
							.4889		
SARCOMA, HISTIOCYTIC	0	0	0	0	1
							.4945		
SCHWANNOMA	1	0	0	0	0	1	1	1	1
						1	1		
joint, tibiofemoral									
# Evaluated	60	60	60	60	60				
SARCOMA, HISTIOCYTIC	0	0	0	0	1
							.4945		
kidneys									
# Evaluated	60	60	60	60	60				
ADENOMA, TUBULAR CELL	0	0	1	0	0	.3409	.	.4026	.
							.2627		
CARCINOMA, TRANSITIONAL CELL	1	0	0	0	0	1	1	1	1
						1	1		
CARCINOMA, TUBULAR CELL	1	0	0	0	2	1	1	1	1
						1	.4917		
LEUKEMIA, LARGE GRANULAR LYMPHOCYTIC	0	0	0	0	1
							.4889		
LIPOSARCOMA	0	1	0	0	0	.6515	.	.	.4713
							.6102		
RENAL MESENCHYMAL TUMOR	0	0	0	0	1
							.4889		
Tub. Cell Adenoma/Carcinoma	1	0	1	0	2	.5673	1	.6463	1
						.4581	.4917		
large intestine, cecum									
# Evaluated	60	60	60	60	60				
LEIOMYOMA	0	1	0	0	0	.6515	.	.	.4713
							.6102		
large intestine, rectum									
# Evaluated	60	60	60	60	60				
FIBROSARCOMA	0	1	0	0	0	.6515	.	.	.4713
							.6102		
SARCOMA, HISTIOCYTIC	0	0	0	0	1
							.4945		

Table A.2.3 (cont.) Incidence and Significance Levels of all Tests on Neoplasms in Male Rats

organ tumor	Incidence					Significance levels			
	PBS	Low	Med	Hi	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1	2vs1
liver									
# Evaluated	60	60	60	60	60				
ADENOMA, HEPATOCELLULAR	0	1	2	1	1	.0829	.2333	.1589	.4713
						.0883	.4889		
CARCINOMA, HEPATOCELLULAR	0	1	0	0	1	.6515	.	.	.4713
						.6102	.4945		
CHOLANGIOCARCINOMA	0	1	0	0	0	.6515	.	.	.4713
						.6102	.		
Hepatocellular Adenoma/Carc.	0	2	2	1	2	.1234	.2333	.1589	.2192
						.1047	.2418		
LEUKEMIA, LARGE GRANULAR LYMPHOCYTIC	0	0	0	0	2
						.	.2362		
SARCOMA, HISTIOCYTIC	0	1	0	0	2	.6541	.	.	.4773
						.6134	.2418		
lung									
# Evaluated	60	60	60	60	60				
CARCINOMA, BRONCH. ALV.	1	0	0	0	0	1	1	1	1
						1	1		
CARCINOMA, TUBULAR CELL	0	0	0	0	1
						.	.4945		
CARCINOMA, UNDIFFERENTIATED	0	0	0	1	0	.1128	.2459	.	.
						.	.		
FIBROSARCOMA	0	1	0	0	0	.6515	.	.	.4713
						.6102	.		
HIBERNOMA	1	0	0	0	0	1	1	1	1
						1	1		
LEUKEMIA, LARGE GRANULAR LYMPHOCYTIC	0	0	0	0	1
						.	.4889		
Pheochromocytoma	0	0	2	0	0	.2567	.	.1652	.
						.0706	.		
SARCOMA, HISTIOCYTIC	0	1	0	0	2	.6541	.	.	.4773
						.6134	.2418		
lymph node, axillary									
# Evaluated	60	60	60	60	60				
FIBROUS HISTIOCYTOMA	0	0	1	0	0	.3409	.	.4026	.
						.2627	.		
SARCOMA, HISTIOCYTIC	0	0	0	0	1
						.	.4945		
lymph node, iliac									
# Evaluated	60	60	60	60	60				
FIBROSARCOMA	0	1	0	0	0	.6515	.	.	.4713
						.6102	.		
lymph node, inguinal									
# Evaluated	60	60	60	60	60				
SCHWANNOMA	0	1	0	0	0	.6515	.	.	.4713
						.6102	.		
lymph node, mediastinal									
# Evaluated	60	60	60	60	60				
CARCINOMA, TUBULAR CELL	0	0	0	0	1
						.	.4945		
FIBROSARCOMA	0	1	0	0	0	.6515	.	.	.4713
						.6102	.		

Table A.2.3 (cont.) Incidence and Significance Levels of all Tests on Neoplasms in Male Rats

organ tumor	Incidence					Significance levels			
	PBS	Low	Med	Hi	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1	2vs1
lymph node, mesenteric									
# Evaluated	60	60	59	58	60				
FIBROSARCOMA	0	1	0	0	0	.6462	.	.	.4713
						.6068	.		
lymph node, renal									
# Evaluated	60	60	60	60	60				
CARCINOMA, TUBULAR CELL	0	0	0	0	1
						.	.4945		
mammary gland									
# Evaluated	60	60	60	60	60				
FIBROADENOMA	2	0	0	1	0	.2974	.5495	1	1
						1	1		
multicentric neoplasm									
# Evaluated	60	60	60	60	60				
LEUKEMIA, LARGE GRANULAR LYMPHOCYTIC	0	0	0	0	2
						.	.2362		
LYMPHOMA	2	1	1	0	0	.7750	1	.7925	.8617
						.6471	1		
SARCOMA, HISTIOCYTIC	0	1	0	0	2	.6541	.	.	.4773
						.6134	.2418		
oropharyngeal tissue									
# Evaluated	60	60	60	60	60				
CARCINOMA, SEBACEOUS CELL	0	1	0	0	0	.6541	.	.	.4773
						.6134	.		
pancreas									
# Evaluated	60	60	60	60	60				
ADENOMA, ISLET CELL	5	5	1	0	2	.9745	1	.9605	.5545
						.9077	.9413		
CARCINOMA, ISLET CELL	2	2	2	0	3	.6971	1	.5451	.6471
						.4015	.4895		
CARCINOMA, TUBULAR CELL	0	0	0	0	1
						.	.4945		
Islet Cell Adenoma/Carc.	7	7	3	0	5	.9736	1	.8659	.5211
						.8044	.8231		
parathyroid glands									
# Evaluated	53	49	51	54	47				
ADENOMA	2	2	2	0	1	.6972	1	.5376	.6063
						.4003	.8484		
pituitary gland									
# Evaluated	60	60	60	60	60				
ADENOMA, PARS DISTALIS	36	47	29	13	36	.9782	.9253	.7103	.0331
						.7910	.5859		
ADENOMA, PARS INTERMEDIA	0	0	1	0	0	.3409	.	.4026	.
						.2627	.		
CARCINOMA, PARS DISTALIS	0	0	0	0	1
						.	.4889		
LEUKEMIA, LARGE GRANULAR LYMPHOCYTIC	0	0	0	0	2
						.	.2362		
SARCOMA, HISTIOCYTIC	0	1	0	0	0	.6541	.	.	.4773
						.6134	.		

Table A.2.3 (cont.) Incidence and Significance Levels of all Tests on Neoplasms in Male Rats

organ tumor	Incidence					Significance levels			
	PBS	Low	Med	Hi	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1	2vs1
preputial glands									
# Evaluated	60	60	60	60	60				
CARCINOMA, SQUAMOUS CELL	1	0	0	0	0	1	1	1	1
						1	1		
PAPILLOMA, SQUAMOUS CELL	0	0	0	1	0	.1061	.2333	.	.
						.	.		
seminal vesicles									
# Evaluated	60	60	60	60	60				
ADENOMA	1	0	0	0	0	1	1	1	1
						1	1		
skeletal muscle									
# Evaluated	60	60	60	60	60				
SCHWANNOMA	0	0	0	0	1
						.	.4945		
skeletal muscle, biceps femoris									
# Evaluated	60	60	60	60	60				
FIBROSARCOMA	0	1	0	0	0	.6515	.	.	.4713
						.6102	.		
LEUKEMIA, LARGE GRANULAR LYMPHOCYTIC	0	0	0	0	1
						.	.4889		
SARCOMA, HISTIOCYTIC	0	0	0	0	1
						.	.4945		
skin									
# Evaluated	60	60	60	60	60				
ADENOMA, BASAL CELL	0	0	1	0	0	.3409	.	.4026	.
						.2627	.		
ADENOMA, SEBACEOUS CELL	0	0	0	0	1
						.	.4889		
KERATOACANTHOMA	7	3	4	0	0	.9162	1	.7449	.9342
						.5833	1		
PAPILLOMA, SQUAMOUS CELL	2	3	0	0	0	.9337	1	1	.4450
						.8828	1		
Sq.Cell Adenoma/Carc./Kerato.	8	6	4	0	0	.9661	1	.8169	.7536
						.7274	1		
skin, subcutis									
# Evaluated	60	60	60	60	60				
CARCINOMA, UNDIFFERENTIATED	0	0	0	1	0	.1128	.2459	.	.
						.	.		
FIBROMA	1	4	1	0	1	.7619	1	.6463	.1464
						.5270	.7473		
FIBROSARCOMA	0	1	1	1	2	.0945	.2333	.4026	.4713
						.2515	.2473		
Fibroma/Fibrosarcoma	1	5	2	1	3	.3725	.4153	.3539	.0819
						.3249	.3083		
FIBROUS HISTIOCYTOMA	0	1	3	3	1	.0046	.0148	.0652	.4713
						.0301	.4889		
Fibroma/Fibrosarcoma/Fibrous Histiocytoma	1	6	5	4	4	.0245	.0165	.0396	.0423
						.0490	.1805		
Fibrosarcoma/Sarcoma	0	1	1	1	3	.0945	.2333	.4026	.4713
						.2515	.1209		
LIPOMA	1	1	0	0	0	.8803	1	1	.7233
						.8501	1		
SARCOMA, HISTIOCYTIC	0	0	0	0	1
						.	.4945		

Table A.2.3 (cont.) Incidence and Significance Levels of all Tests on Neoplasms in Male Rats

organ tumor	Incidence					Significance levels			
	PBS	Low	Med	Hi	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1	2vs1
skin, subcutis (cont.)									
# Evaluated	60	60	60	60	60				
SCHWANNOMA	0	1	1	0	0	.4024	.	.4026	.4713
						.2515	.		
small intestine, duodenum									
# Evaluated	60	60	60	60	60				
ADENOCARCINOMA	0	1	0	0	0	.6515	.	.	.4713
						.6102	.		
small intestine, jejunum									
# Evaluated	60	60	60	60	60				
ADENOCARCINOMA	0	1	0	0	0	.6515	.	.	.4713
						.6102	.		
spinal cord, cervical									
# Evaluated	60	60	60	60	60				
LEUKEMIA, LARGE GRANULAR LYMPHOCYTIC	0	0	0	0	1	.	.4889	.	.
spinal cord, lumbar									
# Evaluated	60	59	60	60	60				
LEUKEMIA, LARGE GRANULAR LYMPHOCYTIC	0	0	0	0	1	.	.4889	.	.
spinal cord, thoracic									
# Evaluated	60	60	60	60	60				
LEUKEMIA, LARGE GRANULAR LYMPHOCYTIC	0	0	0	0	1	.	.4889	.	.
spleen									
# Evaluated	60	60	59	60	60				
LEIOMYOSARCOMA	0	1	0	0	0	.6515	.	.	.4713
						.6102	.		
LEUKEMIA, LARGE GRANULAR LYMPHOCYTIC	0	0	0	0	2	.	.2362	.	.
LIPOSARCOMA	0	0	0	0	1	.	.4945	.	.
						.	.4945	.	.
SARCOMA, HISTIOCYTIC	0	0	0	0	1	.	.4945	.	.
						.	.4945	.	.
stomach, glandular									
# Evaluated	60	60	60	60	60				
FIBROSARCOMA	0	1	0	0	0	.6515	.	.	.4713
						.6102	.		
tail									
# Evaluated	60	60	60	60	60				
PAPILLOMA, SQUAMOUS CELL	0	1	1	0	0	.4024	.	.4026	.4713
						.2515	.		
testes									
# Evaluated	60	60	60	60	60				
ADENOMA, INTERSTITIAL CELL	2	0	2	0	1	.5438	1	.5317	1
						.2817	.8750		
LEUKEMIA, LARGE GRANULAR LYMPHOCYTIC	0	0	0	0	1
						.	.4889	.	.

Table A.2.3 (cont.) Incidence and Significance Levels of all Tests on Neoplasms in Male Rats

organ tumor	Incidence					Significance levels			
	PBS	Low	Med	Hi	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1	2vs1
thyroid gland									
# Evaluated	60	60	60	60	60				
ADENOMA, C-CELL	8	6	3	0	9	.9805	1	.9113	.7380
						.8544	.4798		
ADENOMA, FOLLICULAR CELL	1	1	1	0	1	.6315	1	.6463	.7233
						.4827	.7416		
C-Cell Adenoma/Carcinoma	8	6	3	0	10	.9805	1	.9113	.7380
						.8544	.3765		
CARCINOMA, C-CELL	0	0	0	0	2
						.	.2362		
CARCINOMA, FOLLICULAR CELL	0	2	2	1	0	.1350	.2459	.1589	.2192
						.1047	.		
Foll. Cell Adenoma/Carcinoma	1	2	3	1	1	.2151	.4344	.1852	.4564
						.1132	.7416		
urinary bladder									
# Evaluated	60	60	60	60	60				
CARCINOMA, TRANSITIONAL CELL	0	0	0	1	0	.1061	.2333	.	.
						.	.		
zymbal`s gland									
# Evaluated	59	59	57	57	57				
CARCINOMA, SEBACEOUS CELL	0	0	1	0	0	.3359	.	.4000	.
						.2609	.		

Table A.2.4 Incidence and Significance Levels of all Tests on Neoplasms in Female Rats

organ tumor	Incidence					Significance levels			
	PBS	Low	Med	Hi	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1	2vs1
Lymph Node, Overall									
# Evaluated	59	59	58	59	58				
ADENOCARCINOMA	0	0	0	1	1	.2000	.4030	.	.
						.	.4805		
CARCINOMA, C-CELL	0	0	1	0	0	.4593	.	.4737	.
						.3303	.		
CARCINOMA, SQUAMOUS CELL	0	0	1	0	0	.4593	.	.4737	.
						.3303	.		
Nose									
# Evaluated	60	60	60	60	60				
ASTROCYTOMA	0	1	0	0	0	.7050	.	.	.4533
						.6339	.		
Systemic									
# Evaluated	60	60	60	60	60				
HEMANGIOSARCOMA	0	0	1	0	0	.4638	.	.4744	.
						.3333	.		
Hemangioma/-sarcoma	0	0	1	0	0	.4638	.	.4744	.
						.3333	.		
LYMPHOMA	0	0	1	1	0	.1428	.3971	.4744	.
						.3333	.		
Uterus/Cervix/Vagina									
# Evaluated	60	60	60	60	60				
GRANULAR CELL TUMOR	4	2	6	10	4	.0016	.0164	.3057	.8437
						.1537	.5858		

Table A.2.4 (cont.) Incidence and Significance Levels of all Tests on Neoplasms in Female Rats

organ tumor	Incidence					Significance levels			
	PBS	Low	Med	Hi	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1	2vs1
adrenal glands									
# Evaluated	60	60	60	60	60				
ADENOMA, CORTICAL	2	0	1	0	3	.8487	1	.8599	1
						.7078	.4635		
Pheochromocytoma	3	3	3	1	4	.7679	.8829	.6120	.5690
						.5009	.4568		
brain									
# Evaluated	60	60	60	60	60				
ASTROCYTOMA	0	1	0	0	0	.7050	.	.	.4533
						.6339	.		
CARCINOMA, PARS DISTALIS	6	0	3	1	0	.8281	.9758	.8899	1
						.7152	1		
MIXED GLIOMA	0	1	0	0	0	.7050	.	.	.4533
						.6339	.		
clitoral glands									
# Evaluated	56	60	60	60	57				
CARCINOMA, SQUAMOUS CELL	0	0	1	0	0	.4672	.	.4805	.
						.3364	.		
heart									
# Evaluated	60	60	60	60	60				
ADENOCARCINOMA	1	0	0	0	0	1	1	1	1
						1	1		
SCHWANNOMA	1	1	0	0	1	.9145	1	1	.7045
						.8681	.7339		
kidneys									
# Evaluated	60	60	60	60	60				
ADENOMA, TUBULAR CELL	0	1	0	1	0	.2432	.4058	.	.4533
						.6339	.		
CARCINOMA, TUBULAR CELL	0	0	2	1	1	.1459	.4058	.2282	.
						.1131	.4810		
LIPOSARCOMA	0	0	0	1	0	.1957	.3971	.	.
						.	.		
Tub. Cell Adenoma/Carcinoma	0	1	2	2	1	.0818	.1611	.2282	.4533
						.1381	.4810		
larynx									
# Evaluated	60	60	60	57	60				
CARCINOMA, C-CELL	0	0	1	0	0	.4559	.	.4744	.
						.3333	.		
liver									
# Evaluated	60	60	60	60	60				
ADENOMA, HEPATOCELLULAR	0	1	0	0	0	.7050	.	.	.4533
						.6339	.		
CARCINOMA, ISLET CELL	1	0	0	0	0	1	1	1	1
						1	1		
LEUKEMIA, LARGE GRANULAR LYMPHOCYTIC	0	0	1	0	0	.4638	.	.4744	.
						.3333	.		
lung									
# Evaluated	60	60	60	60	60				
ADENOCARCINOMA	0	1	1	1	0	.2223	.4058	.4744	.4533
						.3095	.		
CARCINOMA, C-CELL	0	0	1	0	0	.4638	.	.4744	.
						.3333	.		
LEUKEMIA, LARGE GRANULAR LYMPHOCYTIC	0	0	1	0	0	.4638	.	.4744	.
						.3333	.		

Table A.2.4 (cont.) Incidence and Significance Levels of all Tests on Neoplasms in Female Rats

organ tumor	Incidence					Significance levels			
	PBS	Low	Med	Hi	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1	2vs1
lymph node, axillary									
# Evaluated	60	59	60	60	60				
ADENOCARCINOMA	0	0	0	1	0	.2014	.4058	.	.
lymph node, cervical									
# Evaluated	60	60	60	60	60				
CARCINOMA, C-CELL	0	0	1	0	0	.4638	.	.4744	.
						.3333	.		
lymph node, inguinal									
# Evaluated	59	60	58	59	58				
ADENOCARCINOMA	0	0	0	0	1
						.	.4805		
lymph node, mandibular									
# Evaluated	60	60	60	60	60				
CARCINOMA, SQUAMOUS CELL	0	0	1	0	0	.4638	.	.4744	.
						.3333	.		
lymph node, mediastinal									
# Evaluated	60	60	60	60	60				
CARCINOMA, C-CELL	0	0	1	0	0	.4638	.	.4744	.
						.3333	.		
mammary gland									
# Evaluated	60	60	60	60	60				
ADENOCARCINOMA	31	29	28	15	25	.9852	.9816	.7180	.4870
						.6907	.8607		
ADENOMA	2	1	2	1	1	.5382	.7903	.6428	.8300
						.4791	.8603		
Adenoma/Adenocarcinoma	32	29	30	15	26	.9893	.9886	.6889	.5675
						.6396	.8841		
FIBROADENOMA	33	21	23	19	22	.7708	.9253	.9423	.9619
						.8710	.9636		
FIBROSARCOMA	1	0	0	0	0	1	1	1	1
						1	1		
multicentric neoplasm									
# Evaluated	60	60	60	60	60				
LEUKEMIA, LARGE GRANULAR LYMPHOCYTIC	0	0	1	0	0	.4638	.	.4744	.
						.3333	.		
LYMPHOMA	0	0	1	1	0	.1428	.3971	.4744	.
						.3333	.		
nerve, trigeminal									
# Evaluated	60	60	60	60	60				
CARCINOMA, PARS DISTALIS	0	0	0	1	0	.1957	.3971	.	.
						.	.		
ovaries									
# Evaluated	60	60	60	60	60				
GRANULOSA CELL TUMOR	0	0	0	2	1	.0394	.1611	.	.
						.	.4810		
oviducts									
# Evaluated	60	60	60	60	60				
LEIOMYOMA	0	0	0	1	0	.2014	.4058	.	.
						.	.		

Table A.2.4 (cont.) Incidence and Significance Levels of all Tests on Neoplasms in Female Rats

organ tumor	Incidence					Significance levels			
	PBS	Low	Med	Hi	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1	2vs1
pancreas									
# Evaluated	60	60	59	60	60				
ADENOMA, ACINAR CELL	0	0	0	1	0	.1957	.3971	.	.
ADENOMA, ISLET CELL	0	3	2	0	1	.7433	.	.2218	.0886
CARCINOMA, ISLET CELL	2	0	1	0	1	.8487	1	.8599	1
Islet Cell Adenoma/Carc.	2	3	3	1	2	.7078	.8652		
						.6620	.7873	.4507	.4106
						.3618	.6630		
parathyroid glands									
# Evaluated	51	51	49	51	48				
ADENOMA	0	1	0	0	1	.6923	.	.	.4462
CARCINOMA, C-CELL	0	1	0	0	1	.6211	.4545		
						.6923	.	.	.4462
						.6211	.4545		
pituitary gland									
# Evaluated	60	60	60	60	60				
ADENOMA, PARS DISTALIS	43	51	45	27	50	.9964	.9592	.2463	.0210
ASTROCYTOMA	0	1	0	0	0	.2873	.0812		
CARCINOMA, PARS DISTALIS	6	0	3	2	0	.7050	.	.	.4533
Pars Distalis Adenoma/Carc.	49	51	48	29	50	.6339	.		
						.6123	.9016	.8899	1
						.7152	1		
						.9992	.9937	.5282	.1755
						.5407	.3934		
skin									
# Evaluated	60	60	60	60	60				
ADENOMA, BASAL CELL	0	0	0	0	1
CARCINOMA, SQUAMOUS CELL	0	0	0	0	1	.	.4810	.	.
KERATOACANTHOMA	0	1	1	0	0	.	.4810	.	.
PAPILLOMA, SQUAMOUS CELL	0	0	0	1	1	.5538	.	.4744	.4459
Sq.Cell Adenoma/Carc./Kerato.	0	1	1	1	2	.3091	.		
						.1957	.3971	.	.
						.	.4810		
						.2166	.3971	.4744	.4459
						.3091	.2282		
skin, subcutis									
# Evaluated	60	60	60	60	60				
FIBROMA	1	1	2	1	0	.3784	.6330	.4520	.6897
FIBROSARCOMA	0	1	4	5	1	.3097	1		
Fibroma/Fibrosarcoma	1	2	6	6	1	.0046	.0098	.0491	.4533
FIBROUS HISTIOCYTOMA	0	0	3	4	0	.0215	.4810		
Fibroma/Fibrosarcoma/Fibr. Histiocytoma	1	2	9	10	1	.0103	.0183	.0405	.4203
Fibrosarcoma/Sarcoma	0	1	4	5	1	.0187	.7275		
						.0053	.0259	.1067	.
						.0370	.		
						.0003	.0007	.0058	.4203
						.0016	.7275		
						.0046	.0098	.0491	.4533
						.0215	.4810		

Table A.2.4 (cont.) Incidence and Significance Levels of all Tests on Neoplasms in Female Rats

organ tumor	Incidence					Significance levels			
	PBS	Low	Med	Hi	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1	2vs1
skin, subcutis (cont.)									
LIPOMA	2	0	0	0	0	1 1	1 1	1	1
RHABDOMYOSARCOMA	0	0	1	0	0	.4638 .3333	.	.4744	.
SCHWANNOMA	1	1	0	0	0	.9145 .8681	1 1	1	.7045
spleen									
# Evaluated	60	60	60	59	60				
LEIOMYOSARCOMA	0	0	0	0	1	.	.4810	.	.
LEUKEMIA, LARGE GRANULAR LYMPHOCYTIC	0	0	1	0	0	.4638 .3333	.	.4744	.
stomach, nonglandular									
# Evaluated	60	60	60	60	60				
PAPILLOMA, SQUAMOUS CELL	0	0	0	1	1	.2014 .	.4058 .4810	.	.
thyroid gland									
# Evaluated	60	60	60	60	60				
ADENOMA, C-CELL	2	4	6	3	6	.2873 .0927	.3122 .1020	.1169	.2545
ADENOMA, FOLLICULAR CELL	0	0	0	0	1	.	.4810	.	.
C-Cell Adenoma/Carcinoma	2	5	9	3	7	.3373 .0183	.3122 .0564	.0218	.1474
CARCINOMA, C-CELL	0	1	3	0	1	.5873 .0567	.	.1067	.4459
CARCINOMA, FOLLICULAR CELL	0	0	0	0	1	.	.4810	.	.
Foll. Cell Adenoma/Carcinoma	0	0	0	0	2	.	.2282	.	.
urinary bladder									
# Evaluated	60	60	60	60	60				
SCHWANNOMA	1	0	0	0	0	1 1	1 1	1	1
uterus with cervix									
# Evaluated	60	60	60	60	60				
GRANULAR CELL TUMOR	2	1	3	8	1	.0008 .2910	.0117 .8652	.4635	.8356
Islet Adenoma/Carcinoma	7	2	4	2	4	.7849 .7025	.9379 .8698	.8493	.9659
POLYP, GLANDULAR	0	0	1	0	0	.4638 .3333	.	.4744	.
POLYP, STROMAL	7	2	4	2	2	.7849 .7025	.9379 .9767	.8493	.9659
SARCOMA, STROMAL	0	0	0	0	2	.	.2345	.	.
SCHWANNOMA	1	0	0	0	0	1 1	1 1	1	1

Table A.2.4 (cont.) Incidence and Significance Levels of all Tests on Neoplasms in Female Rats

organ tumor	Incidence					Significance levels			
	PBS	Low	Med	Hi	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1	2vs1
vagina									
# Evaluated	60	60	60	60	60				
GRANULAR CELL TUMOR	2	1	3	2	3	.2762	.5274	.4393	.8367
						.2694	.4520		
LEIOMYOMA	0	0	0	1	0	.1957	.3971	.	.
						.	.		
zybal's gland									
# Evaluated	60	59	59	59	59				
CARCINOMA, SEBACEOUS CELL	0	0	1	1	1	.1420	.3971	.4675	.
						.3273	.4810		
CARCINOMA, SQUAMOUS CELL	0	0	1	0	0	.4559	.	.4675	.
						.3273	.		

Table A.2.5 Incidence and Significance Levels of all Tests on Neoplasms in Male Mice

organ tumor	Incidence						Significance Levels			
	PBS	Low	Med	Hi	Mnth	I14	trnd1-4/ trnd1-3	4vs1/ 5vs1	3vs1/ 5vs3	2vs1/ 6vs1
Bone Marrow, Ovall										
# Evaluated	68	70	70	70	69	70				
SARCOMA, HISTIOCYTIC	0	1	0	1	0	0	.2483	.4217	.	.5102
							.6667	.	.	.
Injection Site, Ovall										
# Evaluated	70	70	70	70	70	70				
FIBROSARCOMA	0	0	0	1	0	0	.1955	.4167	.	.
						
Lymph Node, Ovall										
# Evaluated	68	64	58	64	65	67				
CARCINOMA, BRONCH. ALV.	1	0	0	0	0	0	1	1	1	1
							1	1	.	1
SARCOMA, HISTIOCYTIC	0	1	0	0	0	0	.7091	.	.	.4894
							.6391	.	.	.
Nose, Ovall										
# Evaluated	69	70	70	70	69	70				
SARCOMA, HISTIOCYTIC	0	0	0	1	0	0	.1955	.4167	.	.
						
Small Intestine, Ovall										
# Evaluated	70	68	69	68	69	70				
ADENOCARCINOMA	0	0	0	0	1	0
							.	.4842	.5000	.
ADENOMA	0	0	0	0	0	1
						5000
Systemic										
# Evaluated	70	70	70	70	70	70				
HEMANGIOSARCOMA	10	7	9	11	10	16	.0731	.2248	.6230	.8456
							.4612	.5191	.5000	.1412
Hemangioma/-sarcoma	10	7	9	11	10	16	.0731	.2248	.6230	.8456
							.4612	.5191	.5000	.1412
LEUKEMIA, GRANULOCYTIC	2	1	0	0	0	1	.9795	1	1	.8750
							.9606	1	.	.8750
LYMPHOMA	1	5	3	3	9	0	.2800	.2042	.2933	.1069
							.3436	.0065	.0601	1

Table A.2.5 (cont.) Incidence and Significance Levels of all Tests on Neoplasms in Male Mice

organ tumor	Incidence						Significance Levels			
	PBS	Low	Med	Hi	Mnth	I14	trnd1-4 trnd1-3	4vs1 5vs1	3vs1 5vs3	2vs1 6vs1
adrenal glands										
# Evaluated	69	70	69	69	69	70				
ADENOMA, CORTICAL	2	1	0	0	2	2	.9801	1	1	.8789
							.9622	.6672	.2473	.6990
ADENOMA, SUBCAPSULAR CELL	3	4	1	0	1	2	.9773	1	.9334	.5114
							.8731	.9334	.7527	.8190
CARC., SUBCAPSULAR CELL	0	1	0	0	0	0	.7247	.	.	.5000
							.6597	.	.	.
bone marrow, femur										
# Evaluated	70	70	70	70	70	70				
SARCOMA, HISTIOCYTIC	0	0	0	1	0	0	.1955	.4167	.	.
						
bone marrow, sternum										
# Evaluated	68	70	70	70	69	70				
SARCOMA, HISTIOCYTIC	0	1	0	0	0	0	.7318	.	.	.5102
							.6667	.	.	.
brain										
# Evaluated	70	70	70	70	70	70				
SCHWANNOMA	1	0	0	0	0	0	1	1	1	1
							1	1	.	1
cavity, abdominal										
# Evaluated	70	70	70	70	70	70				
SARCOMA, HISTIOCYTIC	1	1	0	0	0	0	.9248	1	1	.7525
							.8843	1	.	1
cavity, thoracic										
# Evaluated	70	70	70	70	70	70				
CARCINOMA, BRONCH. ALV.	1	0	0	0	1	0	1	1	1	1
							1	.7421	.5054	1
SARCOMA, HISTIOCYTIC	1	0	0	0	1	0	1	1	1	1
							1	.7369	.5054	1
coagulating glands										
# Evaluated	70	70	70	69	70	70				
ADENOMA	0	0	0	0	0	1
						5000
epididymides										
# Evaluated	70	70	70	70	70	70				
ADENOMA	0	0	0	0	0	1
						5000
ADENOMA, INTERSTIT. CELL	0	0	0	0	0	1
						5000
LEIOMYOMA	1	0	0	0	0	0	1	1	1	1
							1	1	.	1
esophagus										
# Evaluated	70	70	70	70	70	70				
PAPILLOMA, SQUAMOUS CELL	0	0	0	0	0	1
						5000
harderian glands										
# Evaluated	70	70	70	70	70	70				
ADENOCARCINOMA	0	1	0	0	2	0	.7263	.	.	.5000
							.6597	.2318	.2473	.
ADENOMA	9	15	6	3	6	4	.9775	.9383	.8290	.1214
							.8787	.8290	.6207	.9579

Table A.2.5 (cont.) Incidence and Significance Levels of all Tests on Neoplasms in Male Mice

organ tumor	Incidence						Significance Levels			
	PBS	Low	Med	Hi	Mnth	I14	trnd1-4 trnd1-3	4vs1 5vs1	3vs1 5vs3	2vs1 6vs1
head										
# Evaluated	70	70	70	70	70	70				
SCHWANNOMA	1	0	0	0	0	0	1	1	1	1
							1	1	.	1
heart										
# Evaluated	70	70	69	69	70	70				
CARCINOMA, BRONCH. ALV.	1	0	0	0	0	0	1	1	1	1
							1	1	.	1
SARCOMA, HISTIOCYTIC	1	0	0	0	1	0	1	1	1	1
							1	.7369	.5109	1
kidneys										
# Evaluated	70	70	70	70	70	70				
CARCINOMA, BRONCH. ALV.	1	0	0	0	0	0	1	1	1	1
							1	1	.	1
CARCINOMA, TUBULAR CELL	1	0	0	0	0	0	1	1	1	1
							1	1	.	1
SARCOMA, HISTIOCYTIC	1	0	0	0	0	0	1	1	1	1
							1	1	.	1
liver										
# Evaluated	70	70	70	70	70	70				
ADENOMA, HEPATOCELLULAR	13	8	10	11	10	22	.2086	.5014	.7835	.9366
							.6159	.7997	.6196	.0832
CARCINOMA, HEPATOCELLULAR	1	2	8	2	1	4	.2512	.3747	.0153	.5000
							.0038	.7366	.9983	.1938
FIBROSARCOMA	0	0	1	0	0	0	.4556	.	.4896	.
							.3241	.	1	.
Hepato. Adenoma/-carc.	13	10	18	12	11	25	.1826	.3964	.1937	.8421
							.0767	.7218	.9500	.0303
SARCOMA, HISTIOCYTIC	1	2	0	0	1	0	.9169	1	1	.5075
							.8418	.7369	.5054	1
lung										
# Evaluated	70	70	70	70	70	70				
ADENOMA, BRONCH. ALV.	16	15	10	11	18	8	.5724	.6826	.9226	.7110
							.8968	.4169	.0844	.9805
Bonch.-Alv. Adenoma/ Carcinoma	24	18	13	14	23	11	.7206	.8683	.9856	.9179
							.9709	.6520	.0574	.9968
CARCINOMA, BRONCH. ALV.	10	3	6	3	6	3	.8059	.9605	.8833	.9910
							.7199	.8918	.6356	.9910
CARCINOMA, HEPATO.	0	0	2	0	0	0	.4191	.	.2371	.
							.1035	.	1	.
FIBROSARCOMA	0	0	0	1	0	0	.1955	.4167	.	.
						
SARCOMA, HISTIOCYTIC	1	2	0	0	1	0	.9169	1	1	.5075
							.8418	.7369	.5054	1
lymph node, mandibular										
# Evaluated	69	69	67	68	68	69				
SARCOMA, HISTIOCYTIC	0	1	0	0	0	0	.7288	.	.	.5102
							.6643	.	.	.
lymph node, mediastinal										
# Evaluated	70	70	70	70	70	70				
CARCINOMA, BRONCH. ALV.	1	0	0	0	0	0	1	1	1	1
							1	1	.	1

Table A.2.5 (cont.) Incidence and Significance Levels of all Tests on Neoplasms in Male Mice

organ tumor	Incidence						Significance Levels			
	PBS	Low	Med	Hi	Mnth	I14	trnd1-4 trnd1-3	4vs1 5vs1	3vs1 5vs3	2vs1 6vs1
lymph node, mesenteric										
# Evaluated	70	69	67	66	69	70				
SARCOMA, HISTIOCYTIC	0	1	0	0	0	0	.7232	.	.	.5051
							.6597	.	.	.
multicentric neoplasm										
# Evaluated	70	70	70	70	70	70				
HEMANGIOSARCOMA	10	7	9	11	10	16	.0731	.2248	.6230	.8456
							.4612	.5191	.5000	.1412
LEUKEMIA, GRANULOCYTIC	2	1	0	0	0	1	.9795	1	1	.8750
							.9606	1	.	.8750
LYMPHOMA	1	5	3	3	9	0	.2800	.2042	.2933	.1069
							.3436	.0065	.0601	1
SARCOMA, HISTIOCYTIC	2	2	0	2	1	0	.3482	.5593	1	.6988
							.9341	.8671	.5054	1
pancreas										
# Evaluated	70	69	69	69	69	70				
ADENOMA, ISLET CELL	0	1	0	0	1	0	.7247	.	.	.5000
								.6597	.4842	.5000
SARCOMA, HISTIOCYTIC	1	0	0	0	0	0	1	1	1	1
							1	1	.	1
pituitary gland										
# Evaluated	70	69	70	69	70	69				
ADENOMA, PARS DISTALIS	0	1	1	1	0	0	.2220	.4167	.4842	.4948
							.3194	.	1	.
prostate gland										
# Evaluated	70	70	70	69	70	70				
ADENOCARCINOMA	1	0	0	0	0	0	1	1	1	1
							1	1	.	1
skeletal muscle										
# Evaluated	70	70	70	70	70	70				
CARCINOMA, BRONCH. ALV.	1	0	0	0	0	0	1	1	1	1
							1	1	.	1
skin, subcutis										
# Evaluated	70	70	70	70	70	70				
CARCINOMA, BRONCH. ALV.	1	0	0	0	0	0	1	1	1	1
							1	1	.	1
FIBROSARCOMA	0	0	1	4	0	3	.0021	.0291	.4896	.
							.3241	.	1	.1250
SARCOMA, HISTIOCYTIC	2	0	0	1	0	0	.4953	.8016	1	1
							1	1	.	1
Sarcoma/Fibro-/Lipo-	2	0	1	5	0	3	.0072	.1130	.8671	1
							.6913	1	1	.5000
small intestine, duodenum										
# Evaluated	70	69	69	68	69	70				
ADENOCARCINOMA	0	0	0	0	1	0
							.	.4842	.5000	.
ADENOMA	0	0	0	0	0	1
						5000

Table A.2.5 (cont.) Incidence and Significance Levels of all Tests on Neoplasms in Male Mice

organ tumor	Incidence						Significance Levels			
	PBS	Low	Med	Hi	Mnth	I14	trnd1-4 trnd1-3	4vs1 5vs1	3vs1 5vs3	2vs1 6vs1
stomach, glandular										
# Evaluated	70	69	69	69	70	70				
SARCOMA, HISTIOCYTIC	1	0	0	0	0	0	1	1	1	1
							1	1	.	1
testes										
# Evaluated	70	69	70	70	70	70				
ADENOMA, INTERSTIT. CELL	0	2	4	0	2	1	.6464	.	.0537	.2423
							.0324	.2318	.8933	.5000
SERTOLI CELL TUMOR	0	1	0	0	0	0	.7263	.	.	.5000
							.6597	.	.	.
thyroid gland										
# Evaluated	70	69	67	69	70	70				
CARCINOMA, FOLL. CELL	0	0	0	0	1	0
							.	.4896	.5109	.
urinary bladder										
# Evaluated	70	70	70	70	70	70				
CARCINOMA, TRANS. CELL	0	0	0	0	1	0
							.	.4842	.5000	.
MESENCHYMAL TUMOR	2	0	0	0	0	1	1	1	1	1
							1	1	.	.8789
SARCOMA, HISTIOCYTIC	0	1	0	0	0	0	.7278	.	.	.5051
							.6621	.	.	.

Table A.2.6 Incidence and Significance Levels of all Tests on Neoplasms in Female Mice

organ tumor	Incidence						Significance Levels			
	PBS	Low	Med	Hi	Mnth	I14	trnd1-4 trnd1-3	4vs1 5vs1	3vs1 5vs3	2vs1 6vs1
Injection Site, Ovall										
# Evaluated	70	70	70	70	70	70				
SARCOMA, HISTIOCYTIC	0	0	1	0	0	0	.4444	.	.4940	.
							.3254	.	1	.
Lymph Node, Ovall										
# Evaluated	64	66	61	62	63	60				
ADENOCARCINOMA	0	1	0	0	0	0	.7203	.	.	.5062
							.6610	.	.	.
LEIOMYOSARCOMA	1	0	0	0	0	0	1	1	1	1
							1	1	.	1
SARCOMA, HISTIOCYTIC	2	1	0	0	0	0	.9781	1	1	.8796
							.9611	1	.	1
Small Intestine, Ovall										
# Evaluated	70	70	67	67	70	68				
SARCOMA, HISTIOCYTIC	2	0	0	0	0	0	1	1	1	1
							1	1	.	1

Table A.2.6 (cont.) Incidence and Significance Levels of all Tests on Neoplasms in Female Mice

organ tumor	Incidence						Significance Levels			
	PBS	Low	Med	Hi	Mnth	I14	trnd1-4 trnd1-3	4vs1 5vs1	3vs1 5vs3	2vs1 6vs1
Systemic										
# Evaluated	70	70	70	70	70	70				
HEMANGIOMA	1	0	1	1	0	2	.2832 .5359	.6366 1	.7346 1	1 .5787
HEMANGIOSARCOMA	2	8	6	11	9	2	.0025 .2073	.0012 .0416	.1250 .4075	.0482 .7660
Hemangioma/-sarcoma	3	8	7	12	9	4	.0024 .1879	.0022 .0940	.1471 .5272	.1050 .6163
LEUKEMIA, GRANULOCYTIC	0	2	2	0	3	0	.6210 .1736	.	.2410 .5652	.2588 .
LYMPHOMA	13	15	6	7	7	9	.8357 .9802	.8095 .9706	.9790 .5518	.4650 .9596
adrenal glands										
# Evaluated	70	70	69	70	70	70				
ADENOMA, CORTICAL	0	0	0	0	1	1	.	.5227	.5349	.5579
ADENOMA, SUBCAPSULAR CELL	4	1	3	0	2	1	.9061 .5382	1 .9181	.7737 .8536	.9741 .9853
PHEOCHROMOCYTOMA	0	0	0	0	1	1	.	.5227	.5349	.5579
SARCOMA, HISTIOCYTIC	1	0	0	0	0	0	1 1	1 1	1 .	1 1
brain										
# Evaluated	69	70	69	70	70	70				
CARCINOMA, PARS DISTALIS	1	1	0	0	0	0	.9250 .8889	1 1	1 .	.7588 1
cavity, abdominal										
# Evaluated	70	70	70	70	70	70				
ADENOCARCINOMA	0	1	0	0	0	0	.7237 .6640	.	.	.5059 .
FIBROSARCOMA	0	0	0	0	0	15579
LEIOMYOSARCOMA	1	0	0	0	0	0	1 1	1 1	1 .	1 1
LIPOSARCOMA	0	0	0	0	0	15579
SARCOMA, HISTIOCYTIC	3	1	0	0	0	0	.9944 .9877	1 1	1 .	.9419 1
cavity, thoracic										
# Evaluated	70	70	70	70	70	70				
LIPOSARCOMA	0	0	0	0	0	15579
OSTEOSARCOMA	0	0	1	0	0	0	.4408 .3200	.	.4878 1	. .
SARCOMA, HISTIOCYTIC	2	1	1	0	0	0	.8833 .7456	1 1	.8705 1	.8794 1
gallbladder										
# Evaluated	68	68	69	66	69	68				
LEIOMYOMA			0	0	0	0	1 0	.	.5233	.5294 .

Table A.2.6 (cont.) Incidence and Significance Levels of all Tests on Neoplasms in Female Mice

organ tumor	Incidence						Significance Levels			
	PBS	Low	Med	Hi	Mnth	I14	trnd1-4	4vs1	3vs1	2vs1
							trnd1-3	5vs1	5vs3	6vs1
harderian glands										
# Evaluated	70	70	68	70	70	70				
ADENOCARCINOMA	1	3	0	0	3	1	.9335	1	1	.3169
							.8526	.3520	.1584	.8072
ADENOMA	1	3	3	4	4	3	.0547	.0857	.2988	.3169
							.2360	.2099	.5649	.4088
SARCOMA, HISTIOCYTIC	0	1	0	0	0	0	.7219	.	.	.5059
							.6613	.	.	.
head										
# Evaluated	70	70	70	70	70	70				
FIBROSARCOMA	0	0	0	1	0	0	.1830	.4000	.	.
						
heart										
# Evaluated	69	70	69	70	70	70				
SARCOMA, HISTIOCYTIC	2	1	0	0	0	0	.9800	1	1	.8838
							.9639	1	.	1
kidneys										
# Evaluated	70	70	69	70	70	70				
SARCOMA, HISTIOCYTIC	2	2	0	0	0	0	.9704	1	1	.7006
							.9355	1	.	1
larynx										
# Evaluated	70	70	70	68	66	69				
SARCOMA, HISTIOCYTIC	0	1	0	0	0	0	.7237	.	.	.5059
							.6640	.	.	.
liver										
# Evaluated	70	70	68	67	70	69				
ADENOCARCINOMA	0	1	0	0	0	0	.7219	.	.	.5059
							.6640	.	.	.
ADENOMA, HEPATOCELLULAR	4	0	5	11	5	23	<0.0001	.0095	.4844	1
							.1623	.5722	.7148	.0003
CARCINOMA, HEPATOCELLULAR	0	0	0	2	0	2	.0306	.1496	.	.
						3086
Hepato. Adenoma/-carc.	4	0	5	12	5	25	<0.0001	.0044	.4844	1
							.1623	.5722	.7148	.0001
SARCOMA, HISTIOCYTIC	2	2	1	1	1	0	.5986	.7746	.8657	.7006
							.7498	.8913	.7866	1
lung										
# Evaluated	70	70	70	70	70	70				
ADENOCARCINOMA	0	0	1	0	1	0	.4444	.	.4940	.
							.3254	.5227	.7808	.
ADENOMA, BRONCH. ALV.	10	9	17	11	9	8	.0955	.2185	.0942	.7163
							.0346	.7818	.9905	.8974
Bronch.-Alv. Adenoma/ Carcinoma	15	12	20	14	13	10	.1497	.3510	.2181	.8379
							.0928	.8433	.9784	.9727
CARCINOMA, BRONCH. ALV.	5	4	4	3	5	5	.4986	.6850	.7465	.7583
							.6238	.6862	.5722	.7641
FIBROSARCOMA	0	0	0	0	0	1
						5579
LEIOMYOSARCOMA	1	0	0	0	0	0	1	1	1	1
							1	1	.	1
SARCOMA, HISTIOCYTIC	3	2	0	0	0	0	.9905	1	1	.8270
							.9749	1	.	1

Table A.2.6 (cont.) Incidence and Significance Levels of all Tests on Neoplasms in Female Mice

organ tumor	Incidence						Significance Levels			
	PBS	Low	Med	Hi	Mnth	I14	trnd1-4 trnd1-3	4vs1 5vs1	3vs1 5vs3	2vs1 6vs1
lymph node, iliac										
# Evaluated	70	70	70	70	70	70				
SARCOMA, HISTIOCYTIC	1	1	0	0	0	0	.9250 .8889	1 1	1 .	.7588 1
lymph node, inguinal										
# Evaluated	70	69	69	70	70	69				
ADENOCARCINOMA	0	1	0	0	0	0	.7237 .66405059 .
lymph node, mediastinal										
# Evaluated	70	70	69	70	70	70				
SARCOMA, HISTIOCYTIC	1	0	0	0	0	0	1 1	1 1	1 .	1 1
lymph node, mesenteric										
# Evaluated	67	69	65	66	66	67				
SARCOMA, HISTIOCYTIC	2	0	0	0	0	0	1 1	1 1	1 .	1 1
lymph node, renal										
# Evaluated	70	70	70	70	70	70				
LEIOMYOSARCOMA	1	0	0	0	0	0	1 1	1 1	1 .	1 1
SARCOMA, HISTIOCYTIC	1	0	0	0	0	0	1 1	1 1	1 .	1 1
mammary gland										
# Evaluated	69	70	70	70	70	70				
ADENOACANTHOMA	0	0	1	0	0	0	.4444 .3254	. .	.4940 1	. .
ADENOCARCINOMA	2	4	2	2	1	4	.4661 .6147	.5274 .8954	.6830 .8994	.3493 .4552
ADENOMA	0	0	0	0	0	1
Adenoma/-carcinoma	2	4	2	2	1	5	.4661 .6147	.5274 .8954	.6830 .8994	.3493 .3248
multicentric neoplasm										
# Evaluated	70	70	70	70	70	70				
HEMANGIOMA	1	0	1	1	0	2	.2832 .5359	.6366 1	.7346 1	1 .5787
HEMANGIOSARCOMA	2	8	6	11	9	2	.0025 .2073	.0012 .0416	.1250 .4075	.0482 .7660
LEUKEMIA, GRANULOCYTIC	0	2	2	0	3	0	.6210 .1736	. .1428	.2410 .5652	.2588 .
LYMPHOMA	13	15	6	7	7	9	.8357 .9802	.8095 .9706	.9790 .5518	.4650 .9596
SARCOMA, HISTIOCYTIC	4	2	2	1	1	2	.7786 .7736	.9261 .9768	.8879 .8994	.9041 .9384

Table A.2.6 (cont.) Incidence and Significance Levels of all Tests on Neoplasms in Female Mice

organ tumor	Incidence						Significance Levels			
	PBS	Low	Med	Hi	Mnth	I14	trnd1-4 trnd1-3	4vs1 5vs1	3vs1 5vs3	2vs1 6vs1
Ovaries										
# Evaluated	69	70	68	69	69	70				
CYSTADENOMA	2	4	1	3	0	4	.2605	.3225	.8703	.3610
							.7936	1	1	.4676
GRANULOSA CELL TUMOR	1	1	0	0	0	0	.9266	1	1	.7648
							.8907	1	.	1
LEIOMYOSARCOMA	1	0	0	0	0	0	1	1	1	1
							1	1	.	1
LUTEOMA	1	0	0	1	1	0	.3286	.6400	1	1
							1	.7808	.5412	1
SARCOMA, HISTIOCYTIC	1	1	0	0	0	0	.9240	1	1	.7588
							.8871	1	.	1
oviducts										
# Evaluated	70	70	70	70	70	70				
SARCOMA, HISTIOCYTIC	1	0	0	0	0	0	1	1	1	1
							1	1	.	1
pancreas										
# Evaluated	68	69	68	67	68	67				
ADENOMA, ISLET CELL	2	0	0	0	0	0	1	1	1	1
							1	1	.	1
CARCINOMA, ISLET CELL	1	0	0	0	0	0	1	1	1	1
							1	1	.	1
Islet/Acinar Cell Ade- Noma/Carcinoma	3	0	0	0	0	0	1	1	1	1
							1	1	.	1
LEIOMYOSARCOMA	1	0	0	0	0	0	1	1	1	1
							1	1	.	1
LIPOSARCOMA	0	0	0	0	0	1
						5532
SARCOMA, HISTIOCYTIC	1	0	0	0	0	0	1	1	1	1
							1	1	.	1
pituitary gland										
# Evaluated	66	67	69	68	70	69				
ADENOMA, PARS DISTALIS	7	4	8	4	6	6	.4831	.7564	.5000	.9102
							.2794	.8185	.8844	.8565
ADENOMA, PARS INTERMEDIA	0	1	0	1	0	0	.2295	.3971	.	.5060
							.6667	.	.	.
CARCINOMA, PARS DISTALIS	1	1	0	0	0	0	.9266	1	1	.7590
							.8907	1	.	1
Pars Distalis Adenoma/ Carcinoma	8	5	8	4	6	6	.6079	.8275	.6094	.8957
							.4053	.8844	.8844	.9127
skeletal muscle										
# Evaluated	70	70	70	70	70	70				
OSTEOSARCOMA	0	0	0	0	1	0
							.	.5227	.5349	.
skeletal muscle, diaphragm										
# Evaluated	70	70	70	70	70	70				
LEIOMYOSARCOMA	1	0	0	0	0	0	1	1	1	1
							1	1	.	1

Table A.2.6 (cont.) Incidence and Significance Levels of all Tests on Neoplasms in Female Mice

organ tumor	Incidence						Significance Levels			
	PBS	Low	Med	Hi	Mnth	I14	trnd1-4 trnd1-3	4vs1 5vs1	3vs1 5vs3	2vs1 6vs1
skin										
# Evaluated	70	70	70	70	70	70				
CARCINOMA, SQUAMOUS CELL	0	0	0	0	2	0
							.	.2760	.2890	.
HAIR FOLLICLE TUMOR	0	0	0	0	1	1
							.	.5227	.5349	.5579
skin, subcutis										
# Evaluated	70	70	70	70	70	70				
FIBROSARCOMA	0	1	0	0	1	1	.7237	.	.	.5059
							.6640	.5227	.5349	.5579
FIBROUS HISTIOCYTOMA	0	1	0	0	0	0	.7237	.	.	.5059
							.6640	.	.	.
Fib. Histiocytoma/Fibro-sarcoma	0	2	0	0	1	1	.7670	.	.	.2588
							.6560	.5227	.5349	.5579
LIPOSARCOMA	0	0	0	0	0	1
						5579
OSTEOMA	0	0	0	0	0	1
						5625
SARCOMA, HISTIOCYTIC	1	0	0	1	0	0	.3335	.6435	1	1
							1	1	.	1
Sarcoma/Fibro-/Lipo-	1	1	0	1	1	2	.4159	.6435	1	.7588
							.8889	.7751	.5349	.5874
small intestine, duodenum										
# Evaluated	70	70	67	67	70	68				
SARCOMA, HISTIOCYTIC	2	0	0	0	0	0	1	1	1	1
							1	1	.	1
spleen										
# Evaluated	70	70	69	63	67	68				
SARCOMA, HISTIOCYTIC	1	0	1	0	1	0	.6805	1	.7407	1
							.5394	.7698	.7815	1
stomach, glandular										
# Evaluated	70	70	69	68	70	69				
SARCOMA, HISTIOCYTIC	1	0	0	0	0	0	1	1	1	1
							1	1	.	1
stomach, nonglandular										
# Evaluated	70	70	69	68	70	69				
CARCINOMA, SQUAMOUS CELL	0	0	0	0	0	1
						5579
LEIOMYOSARCOMA	1	0	0	0	0	0	1	1	1	1
							1	1	.	1
PAPILLOMA, SQUAMOUS CELL	0	0	1	0	0	0	.4371	.	.4878	.
							.3200	.	1	.
SARCOMA, HISTIOCYTIC	1	0	0	0	0	0	1	1	1	1
							1	1	.	1
tail										
# Evaluated	70	70	70	70	70	70				
SARCOMA, HISTIOCYTIC	0	0	0	0	0	1
						5579
thymus										
# Evaluated	68	63	60	54	60	59				
SARCOMA, HISTIOCYTIC	2	0	0	0	0	0	1	1	1	1
							1	1	.	1

Table A.2.6 (cont.) Incidence and Significance Levels of all Tests on Neoplasms in Female Mice

organ tumor	Incidence						Significance Levels			
	PBS	Low	Med	Hi	Mnth	I14	trnd1-4 trnd1-3	4vs1 5vs1	3vs1 5vs3	2vs1 6vs1
thyroid gland										
# Evaluated	70	70	69	70	69	70				
ADENOMA, FOLLICULAR CELL	2	0	0	0	0	0	1	1	1	1
CARCINOMA, FOLL. CELL	0	0	0	0	1	1
Foll. Cell Adenoma/Carc.	2	0	0	0	1	1	1	1	1	1
							1	.8917	.5294	.9171
tongue										
# Evaluated	70	70	70	70	70	70				
CARCINOMA, SQUAMOUS CELL	0	0	0	0	1	0
							.	.5227	.5349	.
urinary bladder										
# Evaluated	70	70	70	70	70	70				
SARCOMA, HISTIOCYTIC	2	0	0	0	1	0	1	1	1	1
							1	.8954	.5349	1
uterus with cervix										
# Evaluated	70	70	70	70	70	70				
ADENOCARCINOMA	0	0	0	0	1	0
LEIOMYOMA	1	2	3	2	4	0	.2127	.3499	.2988	.5089
LEIOMYOSARCOMA	4	1	4	0	0	2	.1960	.2174	.5769	1
POLYP, GLANDULAR	1	2	2	1	0	1	.8822	1	.6302	.9724
POLYP, STROMAL	4	6	4	4	3	9	.3561	1	1	.9384
Polyp, Stromal+Glandular	5	8	6	5	3	10	.4346	.6330	.4909	.5089
SARCOMA, HISTIOCYTIC	4	2	1	0	1	1	.3629	1	1	.8072
SARCOMA, STROMAL	1	0	0	2	2	0	.3725	.4230	.6300	.3844
							.5880	.8191	.8280	.2285
							.3712	.3817	.5000	.3058
							.4910	.8944	.9408	.2629
							.9799	1	.9688	.9041
							.9208	.9768	.7808	.9843
							.0863	.3499	1	1
							1	.5426	.2890	1
vagina										
# Evaluated	69	70	70	70	70	70				
LEIOMYOSARCOMA	1	0	0	0	0	0	1	1	1	1
SARCOMA, HISTIOCYTIC	0	0	0	0	1	0	1	1	.	1
							.	.5287	.5349	.

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/s/

STEVEN F THOMSON
01/10/2013
Statistical Carcinogenicity Review

KARL K LIN
01/10/2013
Concur with review



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

STATISTICAL REVIEW AND EVALUATION

CLINICAL STUDIES

NDA/BLA Serial Number: 203568/000

Drug Name: Mipomersen sodium Solution for Injection

Indication(s): Reduce LDL-C, apo B, total cholesterol, non-HDL-C, and Lp(a) in patients with HoFH

Applicant: Genzyme Corporation

Date(s): 03/29/2012

Review Priority: Standard

Biometrics Division: 2

Statistical Reviewer: Japobrata Choudhury, Ph.D.

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Project Manager: Kati Johnson

Keywords:

Link to DBII key Words: Clinical Studies, NDA Review

http://intranetapps.fda.gov/scripts/ob_apps/ob/eWork/uploads/eWork/2009/Keywords-in-DFS.htm

Table of Contents

1. EXECUTIVE SUMMARY	3
2. INTRODUCTION.....	5
2.1 OVERVIEW	6
2.2 DATA SOURCES	10
3. STATISTICAL EVALUATION.....	10
3.1 DATA AND ANALYSIS QUALITY	10
3.2 EVALUATION OF EFFICACY	11
Pivotal Study ISIS 301012-CS5, in patients with HoFH.....	11
Supportive Studies.....	38
3.3 EVALUATION OF SAFETY	42
4. FINDINGS IN SPECIAL/SUBGROUP POPULATIONS	42
4.1 GENDER, RACE, AGE, AND GEOGRAPHIC REGION	42
4.2 OTHER SPECIAL/SUBGROUP POPULATIONS	52
5. SUMMARY AND CONCLUSIONS	54
5.1 STATISTICAL ISSUES AND COLLECTIVE EVIDENCE	60
5.2 CONCLUSIONS AND RECOMMENDATIONS	60
APPENDICES (ADD WHEN NEEDED).....	62
SIGNATURES/DISTRIBUTION LIST (OPTIONAL)	67

1. EXECUTIVE SUMMARY

The proposed indication for mipomersen sodium, an apolipoprotein B synthesis inhibitor, is as an adjunct to maximally tolerated lipid-lowering medications and diet to reduce low density lipoprotein-cholesterol, apolipoprotein B, total cholesterol, non-high density lipoprotein-cholesterol and lipoprotein (a) in patients with homozygous familial hypercholesterolemia.

Orphan Drug Designation (No. 06-2214) for the use of mipomersen for the treatment of HoFH was granted on May 23, 2006. Patients with HoFH are characterized by having elevated plasma cholesterol levels up to 10-fold higher than normal, with untreated LDL-C levels in the range of 650 mg/dL from early childhood (Goldstein, 2001, Science). Major adverse cardiovascular events can occur as early as 18 months of age with premature death due to extremely high lipid burden as early as three years of age (Goldstein, 2001, The Metabolic and Molecular Bases of Inherited Disease).

The mipomersen development programme has been designed to support treatment of HoFH patients, who, despite maximally tolerated lipid-lowering therapy and low-fat diet, remain at very high CHD risk due to life-long exposure to very high LDL-C levels. Because mipomersen inhibits the synthesis of apo B and reduces the secretion of apo B-containing lipoproteins including LDL precursors, it does not depend on hepatic LDLr expression for its hypolipidaemic effect; thus, mipomersen offers a new and much needed approach to lower LDL-C in HoFH patients who do not respond sufficiently to available lipid-lowering therapies.

This submission presents data from a pivotal Phase 3 study in the indicated patient population ([ISIS 301012-CS5](#), in patients with HoFH). Data from 3 supportive Phase 3 studies in patients with related conditions ([MIP003500108](#), in patients with Severe HeFH; [ISIS 301012-CS7](#), in patients with HeFH and CAD; and [ISIS 301012-CS12](#), in patients with hypercholesterolaemia at high risk for CHD) are also presented.

Total Exposure

As of the data cutoff date of 30 November 2011, a total of 811 subjects have been exposed to mipomersen via the SC, IV, and/or oral administration routes. A total of 749 subjects have been exposed to at least 1 SC injection of mipomersen. The overall exposure to the indicated dose of 200 mg SC weekly included 586 subjects, for a total exposure period of 325.6 patient-years. A total of 243 patients received mipomersen at the indicated dose for at least 6 months, 113 patients have been treated for at least 12 months, 75 patients have been treated for at least 18 months, and 54 patients have been treated for at least 24 months.

Primary Results

(ISIS 301012-CS5, in patients with HoFH)
Percent Change in LDL Cholesterol From Baseline to the Primary
Efficacy Time Point (Gravimetric Units) – Full Analysis Set

Time point Statistic	Placebo (N = 17)	Mipomersen (N = 34)
Baseline (mmol/L)		
n	17	34
Mean (SD)	10.37 (3.666)	11.37 (3.588)
Min, Max	4.45, 16.54	4.92, 18.23
PET (mmol/L)		
n	17	34
Mean (SD)	10.06 (3.899)	8.45 (3.142)
Min, Max	3.34, 15.70	1.61, 15.20
Percent change		
n	17	34
Mean (SD)	-3.31 (17.06)	-24.65 (19.86)
Min, Max	-33.4, 43.1	-81.8, 2.1
95% CI	(-12.1, 5.5)	(-31.6,-17.7)
Wilcoxon signed rank test (p-value)	0.323	<0.001
t-test (p-value)		<0.001

For patients with TG <400 mg/dL, LDL-C was obtained using Friedewald's calculation; and for patients with TG ≥400 mg/dL, LDL-C was directly measured by the central laboratory using ultracentrifugation.
CI = confidence interval; Max = maximum; Min = minimum; PET = primary efficacy time point;
SD = standard deviation.

Note: The p-values in the last column of the above Table are for the difference between the two treatments.

Conclusion

Based on this reviewer's own parametric (including SAS Generalized Linear Model program) and nonparametric (Wilcoxon's two-sample test) analyses and the sponsor's results, efficacy results are highly significant showing the efficacy of mipomersen with respect to LDL-C over 26 weeks ($p < .001$). Statistical results for secondary endpoints Apo B, non-HDL, and TC were also highly significant ($p < .001$).

There were no imbalances with respect to baseline factors/covariates. In the pooled analysis, gender by treatment interaction was statistically significant ($p < 0.001$). The treatment effect in females was larger than that seen in males. More detailed discussions are in Section 5.

There are no data to show the efficacy of the drug for the possible long-term treatment.

Labeling

L_p (a) included in the labeling was not pre-specified as a secondary efficacy variable.

2. INTRODUCTION

Note: Tables and Figures presented in this document are referenced by “below” or “above”. Those referenced with an extended numbering system are in the NDA Study Report. Unless mentioned otherwise, the source of all information is the sponsor’s submission. The reviewer’s interpretations, comments, or conclusions are clearly identified under notes, comments, or separate sections.

Appendix II is a list of abbreviations.

-----INDICATIONS AND USAGE-----

Mipomersen is an apolipoprotein B synthesis inhibitor indicated as an adjunct to maximally tolerated lipid-lowering medications and diet to reduce low density lipoprotein-cholesterol, apolipoprotein B, total cholesterol, non-high density lipoprotein-cholesterol and lipoprotein (a) in patients with homozygous familial hypercholesterolemia.

Mipomersen sodium is a 20-base, synthetic, oligonucleotide sodium salt and a first-in-class antisense inhibitor of apolipoprotein B (apo B) synthesis.

HoFH is a population with the most extreme form of familial hypercholesterolemia. Orphan Drug Designation (No. 06-2214) for the use of mipomersen for the treatment of HoFH was granted on May 23, 2006. Patients with HoFH are characterized by having elevated plasma cholesterol levels up to 10-fold higher than normal, with untreated LDLC levels in the range of 650 mg/dL from early childhood (Goldstein, 2001, Science). Major adverse cardiovascular events can occur as early as 18 months of age with premature death due to extremely high lipid burden as early as three years of age (Goldstein, 2001, The Metabolic and Molecular Bases of Inherited Disease).

Mipomersen inhibits expression of the apo B-100 gene by sequence-specific hybridisation, or binding, to a complementary sequence on the messenger ribonucleic acid (mRNA) through Watson-Crick base-pair interactions. This results ultimately in selective degradation of the mRNA through one of several possible enzyme-mediated pathways or, alternatively, destabilisation or disruption of the mRNA's metabolism and function through binding alone.

Mipomersen is a phosphorothiolated oligonucleotide and therefore differs from naturally occurring oligonucleotides by substitution of the phosphate diester internucleotide

linkage by a phosphorothioate diester. Other modifications are methylation of the cytosine bases at the 5-position and substitution in the 2'-position with a 2-methoxyethyl moiety for 10 of 20 nucleotides. These modifications result in a compound that is more stable in vivo and more active than unmodified oligonucleotides.

Mipomersen Development

(b) (4)

2.1 Overview

Summary of Major Clinical Efficacy Studies

Study No. Phase	Design Dose, Route, Regimen and Duration	Study Objective, Primary Endpoint	Patients: Diagnosis No. Planned No. Analysed	Demography: Gender (M/F) Median Age (range)
Pivotal Study				
ISIS 301012-CS5 Phase 3	Randomised, double-blind, placebo-controlled 200 mg mipomersen (160 mg for patients weighing <50 kg) or placebo weekly SC for 26 weeks	Efficacy and safety % change in LDL-C from baseline to PET, placebo vs. mipomersen	HoFH Planned: 50 Analysed: 51 (17 placebo, 34 mipomersen)	41.2%/58.8% placebo; 44.1%/55.9% mipomersen 33.0 years (12-53 years) placebo; 30.4 years (14-53 years) mipomersen
Supportive Studies				
MIPO3500108 Phase 3	Randomised, double-blind, placebo-controlled 200 mg mipomersen or placebo weekly SC for 26 weeks	Efficacy and safety % change in LDL-C from baseline to PET, placebo vs. mipomersen	Severe hypercholesterolaemia ^a Planned: 51 to 75 Analysed: 58 (19 placebo; 39 mipomersen)	36.8%/63.2% placebo; 46.2%/53.8% mipomersen 52 years (18-66 years) placebo; 51 years (21-77 years) mipomersen

ISIS 301012-CS7 Phase 3	Randomised, double-blind, placebo-controlled 200 mg mipomersen or placebo weekly SC for 26 weeks	Efficacy and safety % change in LDL-C from baseline to PET, placebo vs. mipomersen	HeFH Planned: 100 to 125 Analysed: 124 (41 placebo; 83 mipomersen)	68.3%/31.7% placebo; 60.2%/39.8% mipomersen 56 years (47-62 years) placebo; 55 years (51-63 years) mipomersen
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Summary of Major Clinical Efficacy Studies

Study No. Phase	Design Dose, Route, Regimen and Duration	Study Objective, Primary Endpoint	Patients: Diagnosis No. Planned No. Analysed	Demography: Gender (M/F) Median Age (range)
ISIS 301012-CS12 Phase 3	Randomised, double-blind, placebo-controlled 200 mg mipomersen or placebo weekly SC for 26 weeks	Efficacy and safety % change in LDL-C from baseline to PET, placebo vs. mipomersen	High-risk HC Planned: 180 Analysed: 158 (53 placebo; 105 mipomersen)	55.8%/44.2% placebo; 49.5%/50.5% mipomersen 59 years (37-79 years) placebo; 60 years (36-81 years) placebo

HC, hypercholesterolaemia; HeFH, heterozygous familial hypercholesterolaemia; HoFH, homozygous familial hypercholesterolaemia; LDL-C, low density

lipoprotein cholesterol; PET, primary efficacy time point, defined as the post-baseline visit closest to 14 days after the last dose of study treatment for which LDL-C was assessed; SC, subcutaneous; UK, United Kingdom

^a As discussed in [Section 1.4.1.2](#), the patient population from MIPO3500108 is the same population now designated as the Severe HeFH population, although this terminology was not used in the protocol.

Source: [ISIS301012-CS5 CSR](#); [MIPO3500108 CSR](#); [ISIS301012-CS7 CSR](#); [ISIS301012-CS12 CSR](#)

The pivotal Study ISIS301012-CS5 was an international, multicentre study conducted in a total of 9 study centres in Africa (South Africa), Asia (Singapore and Taiwan), Europe (United Kingdom), North America (Canada, United States), and South America (Brazil).

Patient population and baseline findings: Fifty-one patients were randomised: 34 patients were randomised to mipomersen treatment and 17 patients were randomised to placebo treatment. Four patients in the mipomersen group and 2 patients in the placebo group weighed <50 kg, and, therefore, received the lower dose of 160 mg mipomersen or matching placebo. All other patients received a dose of 200 mg or matching placebo. Six (11.8%) patients, all in the mipomersen group, discontinued from the study during the treatment period.

Of the 51 patients, 29 (56.9%) were female. The majority of patients were White (38 [74.5%]). The median age was 27 years for patients in the mipomersen group and 38 years for patients in the placebo group. Seven (20.6%) patients in the mipomersen group and 3 (17.6%) patients in the placebo group were tobacco users. Fourteen (41.2%) patients in the mipomersen group and 6 (35.3%) patients in the placebo group were current users of alcohol.

Seven (20.6%) patients in the mipomersen group and 1 (5.9%) patient in the placebo group had metabolic syndrome at baseline. With the exception of baseline metabolic syndrome, the treatment groups were comparable with respect to demographics and baseline characteristics.

The sponsor's further statements:

- Data from ISIS 301012-CS5, in patients with HoFH, demonstrated that treatment with mipomersen 200 mg once weekly via subcutaneous (SC) injection resulted in clinically meaningful and statistically significant decreases in LDL-C compared to placebo in patients with HoFH (-24.7% versus -3.3%; $p < 0.001$).
- Data from ISIS 301012-CS5, in patients with HoFH, also demonstrated that treatment with mipomersen 200 mg once weekly via SC injection resulted in clinically meaningful and statistically significant decreases in apo B-100 compared to placebo (-26.8% versus -2.5%; $p < 0.001$), with corresponding reductions in LDL-C and Lp(a). This clearly demonstrates that mipomersen has the intended effect of targeting reduction in the synthesis of apo B-100.
- Mipomersen treatment in patients with HoFH demonstrated clinically meaningful and statistically significant decreases on all other measured atherogenic lipoproteins and their components, including VLDL-C, TC, and non-HDL-C levels as compared to placebo-treated patients, consistent with mipomersen's mechanism of action targeting reduction in synthesis of all apo B-100-containing atherogenic proteins.
- In HoFH patients where the Lp(a) at baseline was, appropriately, highest among the pivotal study populations, mipomersen-treatment patients demonstrated a statistically significant and clinically meaningful decrease in Lp(a) levels as compared to placebo-treated patients. This is further evidence of mipomersen's atherogenic lipoproteins.

The results from the 3 supportive Phase 3 studies demonstrated:

- Atherogenic lipid level reductions across a range of significantly elevated baseline LDL-C levels in patient populations with a high risk of a cardiovascular event and on maximally tolerated therapy. MIPO3500108 was conducted in patients with Severe HeFH; ISIS 301012-CS7 was conducted in patients with HeFH and CAD; and ISIS 301012-CS12 was conducted in patients with hypercholesterolaemia at high risk for CHD. Thus, there is no reason to assume that the efficacy of mipomersen is clinically meaningfully different in non-HoFH populations irrespective of the pretreatment LDL-C level.
- Mipomersen 200 mg administered once weekly via SC injection resulted in a clinically meaningful and statistically significant mean percent reduction in LDL-C compared with placebo.
- Mipomersen-treated patients demonstrated statistically significant and clinically meaningful decreases in apo B-100 as compared to placebo-treated patients. These decreases were of similar magnitude to the corresponding change in LDL-C observed in each study.
- Furthermore, mipomersen-treated patients demonstrated statistically significant and clinically meaningful decreases in Lp(a) compared to placebo.

In all 4 Phase 3 studies (The sponsor's further statements continued):

- □ Mipomersen treatment reached the average maximum effect after approximately 4 to 6 months.
- □ There was no evidence of a decrease in HDL-C levels in mipomersentreated patients.
- □ Both males and females had clinically meaningful and statistically significant reductions in LDL-C. While there were no gender differences observed in HoFH patients, statistically significant differences were noted in Phase 3 studies of patients with Severe HeFH, HeFH and CAD, and patients with hypercholesterolaemia at high risk for CHD. Females tended to have somewhat higher reductions in LDL-C levels than males. Nevertheless, the reductions observed in male patients were clinically meaningful and statistically significant.
- □ Data from an ongoing open-label extension (OLE) study demonstrate that longterm mipomersen treatment results in stable, clinically significant mean percent reductions in LDL-C and apo B up to at least 2 years of therapy. The mean percent reductions in LDL-C and apo B were consistent with those observed during the 26-week, double-blind treatment periods of the index studies.
- □ The efficacy conclusions are further supported by data from Phase 1 and 2 studies.

The pivotal Phase 3 study (ISIS 301012-CS5) and the supportive Phase 3 studies (MIPO3500108, ISIS 301012-CS7, and ISIS 301012-CS12) were randomised, doubleblind, placebo-controlled, parallel-group studies designed to determine the effects of 26 weeks of mipomersen therapy on LDL-C levels in patients not reaching target lipid goals on current lipid-lowering therapy (including maximally tolerated statins; see Section 1.4.2). The studies consisted of a ≤ 4 -week screening period, 26 weeks of treatment, and a 24-week post-treatment follow-up period (unless patients enrolled into an OLE study). The 26-week treatment duration was based on the elimination half-life of mipomersen (observed range of terminal elimination half-life in Phase 3 studies, 22.48 to 50.86 days; see 2.7.2), so that the efficacy endpoint could be evaluated when drug tissue levels were expected to be $\geq 90\%$ of steady-state values.

Eligible patients were randomised in a 2:1 ratio using an Interactive Voice Response System (IVRS) to receive mipomersen 200 mg SC once weekly or placebo SC once weekly, respectively. The 2:1 randomisation ratio was used to increase the number of patients exposed to mipomersen in the small patient populations available for these studies.

Samples for fasting serum lipid panels were taken at screening, prior to the first dose of study drug, at approximately 4-week intervals during the treatment period, and during the post-treatment follow-up period. The primary analysis of efficacy parameters was assessment of the percent change from baseline to the primary efficacy time point (PET, defined as the post-baseline visit closest to 14 days after the last dose of study treatment for which LDL-C was assessed; for full definition, see Section 1.6.3) compared between treatment groups.

Endpoints

The primary efficacy parameter for the Phase 3 studies was the percent change in LDL-C from baseline to PET (the post-baseline visit closest to 14 days after the last dose of study treatment for which LDL-C is assessed).

Reduction in LDL-C was used as the primary efficacy endpoint across all safety and efficacy studies in the mipomersen clinical development programme.

Secondary efficacy parameters included percent changes from baseline to PET in apo B, non-HDL-C, and TC levels; tertiary efficacy parameters included percent change in TG, Lp(a), VLDL-C, LDL/HDL ratio, apo A-I, and HDL-C.

Statistical Analyses

In general, primary, secondary, and tertiary efficacy endpoints were analysed consistently across all Phase 3 studies (pivotal and supportive) as outlined in the [Mipomersen Core Statistical Analysis Plan \(MCSAP\)](#). Efficacy analyses were specified in the MCSAP or in the Study-Specific Statistical Analysis Plans (SSSAPs), completed prior to database lock for the Phase 3 studies. The methods delineated in the MCSAP were deemed best practices and facilitated consistency in the analyses and reporting of the Phase 2 and 3 mipomersen studies. When the analysis approach presented in the MCSAP or SSSAPs deviated from those delineated in the clinical study protocols or, in the case of early-phase studies (in which databases were locked, unblinded, and analysed before writing of the MCSAP) from those originally executed, such differences were clearly outlined and justified in each SSSAP.

Efficacy analyses, corrections for multiple analyses by use of a sequential inferential approach, and sensitivity analyses were performed.

Analysis Set

All efficacy parameters were assessed on the Per-Protocol Set (PPS) and Full Analysis Set (FAS), with the latter being the basis for the primary efficacy analysis. The FAS, which represented the practically-feasible intent-to-treat (ITT) population as delineated in International Conference on Harmonisation (ICH) Guideline E9, consisted of the subset of the Safety Set with a valid baseline and at least one post-baseline LDL-C measure.

2.2 Data Sources

0000 on 'Cdsesub1\evsprod\Nda203568'

3. STATISTICAL EVALUATION

3.1 Data and Analysis Quality

No concerns were raised by any investigations or found by this reviewer.

3.2 Evaluation of Efficacy

Study Design and Endpoints

This was a randomized, double-blind, placebo-controlled study, which consisted of a ≤ 4 -week screening period, 26-week treatment period, and a 24-week post-treatment follow-up period (with the exception of patients who enrolled in the open-label extension study [Study ISIS 301012-CS6]). The screening (pre-treatment) period was the time from when the patient gave informed consent until the time the first dose of study treatment was administered. The treatment period spanned the time during which the study treatment was administered until the later of the primary efficacy timepoint (PET) or 14 days beyond the last day of study treatment administration. The post-treatment follow-up period began the day after the treatment period and ended on the day of the patient's last contact date within the study.

The study population included male and non-pregnant, non-lactating female patients with a diagnosis of HoFH, aged ≥ 12 years, Tanner Stage >2 , with a fasting LDL-C ≥ 130 mg/dL and TG <350 mg/dL at screening.

Eligible patients were randomized in a 2:1 ratio to receive mipomersen or placebo as a subcutaneous (SC) injection, once a week for 26 weeks. Patients were stratified by weight: patients who weighed ≥ 50 kg received 200 mg mipomersen or matching volume of placebo, and patients who weighed ≤ 50 kg received a lower dose of 160 mg mipomersen or matching volume of placebo. Patients who were on stable (≥ 12 weeks) concomitant lipid-lowering therapy at screening were required to remain on the same dose and regimen throughout the study.

The primary endpoint assessment was at Week 28. Following treatment and Week 28 evaluations, eligible patients who tolerated study drug could elect to enroll in an open-label extension study. Patients who were not eligible for, or who elected not to enroll in the open-label extension study, were followed in this study for an additional 22 weeks (24 weeks from administration of the last dose of study drug).

Once written informed consent was obtained, a patient's eligibility for entry into the study was assessed according to the inclusion and exclusion criteria. Patients gave a full medical history, and underwent a full physical examination, including anthropometric and vital sign measurements. A 12-lead electrocardiogram (ECG) tracing was obtained to screen for underlying cardiac disease. Blood and urine samples were collected for clinical laboratory evaluations according to the schedule of events. The patient was counseled by a staff nutritionist or dietician on the Therapeutic Lifestyle Changes approach; this included advice on diet that should have been maintained throughout the course of the study.

Baseline magnetic resonance imaging (MRI) or computed tomography (CT) imaging limited to the liver was collected for all patients prior to administration of the first dose of study drug, and the image was stored for future analysis, if required. Patients reported to the study center after an overnight fast for

□ 10 hours (only water was permitted) for a full serum lipid panel on Day 1. A physical examination was performed and a blood sample for baseline immunoglobulin (Ig) G and mipomersen antibody measurements were drawn prior to study drug administration. A serum pregnancy test was conducted for all female patients of childbearing potential. Blood samples for pharmacokinetic (PK) trough levels were required to be drawn prior to mipomersen administration at all outpatient visits during the treatment period.

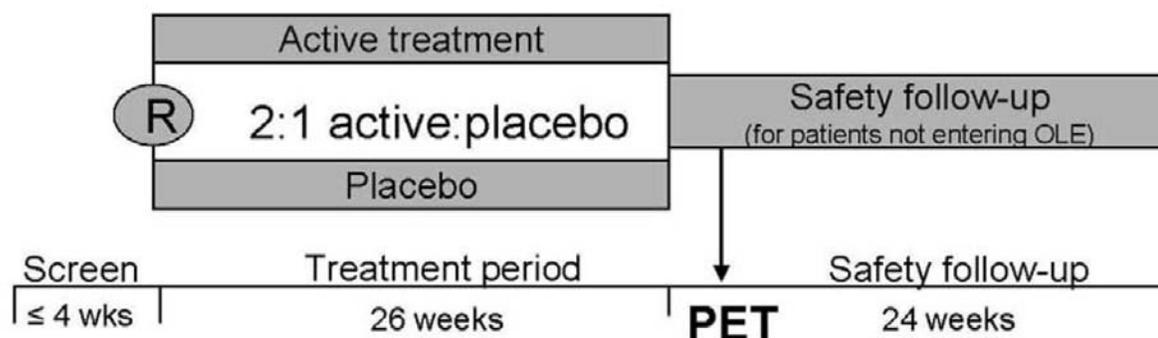
Patients then were randomized to either mipomersen or placebo and received study drug that day. All patients were required to provide a urine sample between 1 and 10 hours after their first dose to measure drug concentration. This measurement was used as a reference in the event that self administration was necessary during the treatment period.

During the treatment period, vital signs and body weight were measured. Prior to study drug administration and according to the study schedule, fasting blood and urine samples for clinical laboratory tests including hematology with differential, chemistry, full serum lipid panel, and urinalysis were performed. Cardiovascular risk markers (hsCRP and LDL particle subclasses) were measured at baseline and post-treatment. Samples for lipid metabolism panels were drawn prior to, during, and post-treatment.

At the Week 28 outpatient visit, patients underwent a full physical examination, including height, body weight, vital sign measurements, and a 12-lead ECG tracing. A blood sample for immunoglobulin (Ig) G and mipomersen antibody measurements was also drawn at the Week 28 outpatient visit.

Blinded safety data were monitored on a real time basis by the Medical Monitor. The Data Safety Monitoring Board reviewed blinded safety tables and listings approximately every 3 months.

Study Design Figure



OLE = open-label extension; PET = primary efficacy time point; R = randomization.

Endpoints

The primary efficacy parameter for the Phase 3 studies was the percent change in LDL-C from baseline to PET (the post-baseline visit closest to 14 days after the last dose of study treatment for which LDL-C is assessed).

Reduction in LDL-C was used as the primary efficacy endpoint across all safety and efficacy studies in the mipomersen clinical development programme.

Secondary efficacy parameters included percent changes from baseline to PET in apo B, non-HDL-C, and TC levels; tertiary efficacy parameters included percent change in TG, Lp(a), VLDL-C, LDL/HDL ratio, apo A-I, and HDL-C.

Patient Disposition, Demographic and Baseline Characteristics

Patient Disposition

Table below summarizes patient disposition for all screened patients. In total, 61 patients were screened and 51 patients were randomized at 9 study centers. For details of patient disposition by study center (Table 14.1.2.2. in CSR).

Thirty-four patients were randomized to mipomersen treatment and 17 patients were randomized to placebo treatment. Four patients in the mipomersen group and 2 patients in the placebo group weighed <50 kg, and therefore received the lower dose of 160 mg mipomersen or matching placebo. All other patients received a dose of 200 mg or matching placebo. The weight of Patient 1523-8649 in the mipomersen group was <50 kg at study entry but increased to >50 kg during the course of the treatment period; however, the patient remained on the 160 mg dose throughout the study.

Six (11.8%) patients, all in the mipomersen group, discontinued from the study during the treatment period: 4 (7.8%) patients discontinued due to an AE, 1 (2.0%) patient was discontinued due to physician decision, and 1 (2.0%) patient withdrew consent. In total, 45 (88.2%) patients completed the treatment period.

Twelve patients entered the post-treatment follow-up period: 1 patient in the placebo group and 11 patients in the mipomersen group. Of these, 1 patient in the placebo group and 5 patients in the mipomersen group discontinued the post-treatment follow-up period. Six (11.8%) patients in the mipomersen group completed the post-treatment follow-up period.

Thirty-nine patients enrolled in the open-label extension study: 16 (94.1%) patients from the placebo group and 23 (67.6%) patients from the mipomersen group.

Patient Disposition – All Screened Patients

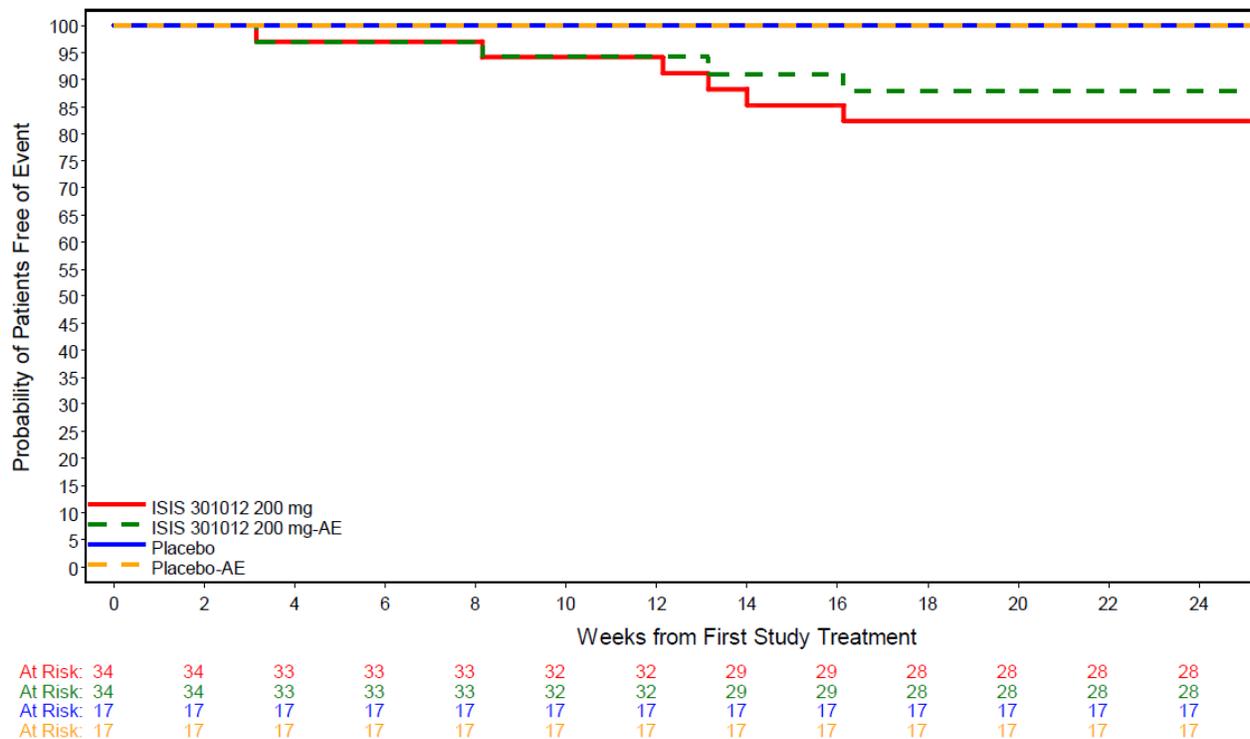
Placebo (N = 17) n (%)	Mipomersen (N = 34) n (%)	Total (N = 51) n (%)
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Screened			61
Screen failures, n (% of screened)			10 (16.4)
Randomized, n (% of screened)	17	34	51 (83.6)
Treated, n (% of randomized)	17 (100.0)	34 (100.0)	51 (100.0)
Completed treatment, n (% of randomized)	17 (100.0)	28 (82.4)	45 (88.2)
Enrolled in open-label extension study	16 (94.1)	23 (67.6)	39 (76.5)
Completed follow-up, n (% of randomized)	0 (0.0)	5 (14.7)	5 (9.8)
Discontinued follow-up, n (% of randomized)	1 (5.9)	0 (0.0)	1 (2.0)
Withdrawal by patient	1 (5.9)	0 (0.0)	1 (2.0)
Discontinued treatment, n (% of randomized)	0 (0.0)	6 (17.6)	6 (11.8)
AE or SAE	0 (0.0)	4 (11.8)	4 (7.8)
Physician decision	0 (0.0)	1 (2.9)	1 (2.0)
Withdrawal by patient	0 (0.0)	1 (2.9)	1 (2.0)
Completed follow-up, n (% of randomized)	0 (0.0)	1 (2.9)	1 (2.0)
Discontinued follow-up, n (% of randomized)	0 (0.0)	5 (14.7)	5 (9.8)
Other	0 (0.0)	1 (2.9)	1 (2.0)
Protocol non-compliance	0 (0.0)	1 (2.9)	1 (2.0)
Withdrawal by patient	0 (0.0)	3 (8.8)	3 (5.9)

AE = adverse event; SAE = serious adverse event.

Source: [Table 14.1.2.1](#)

Time to Treatment Discontinuation, Overall and Due to Adverse Events, Safety Set in ISIS 301012-CS5



Demographic and Baseline Characteristics

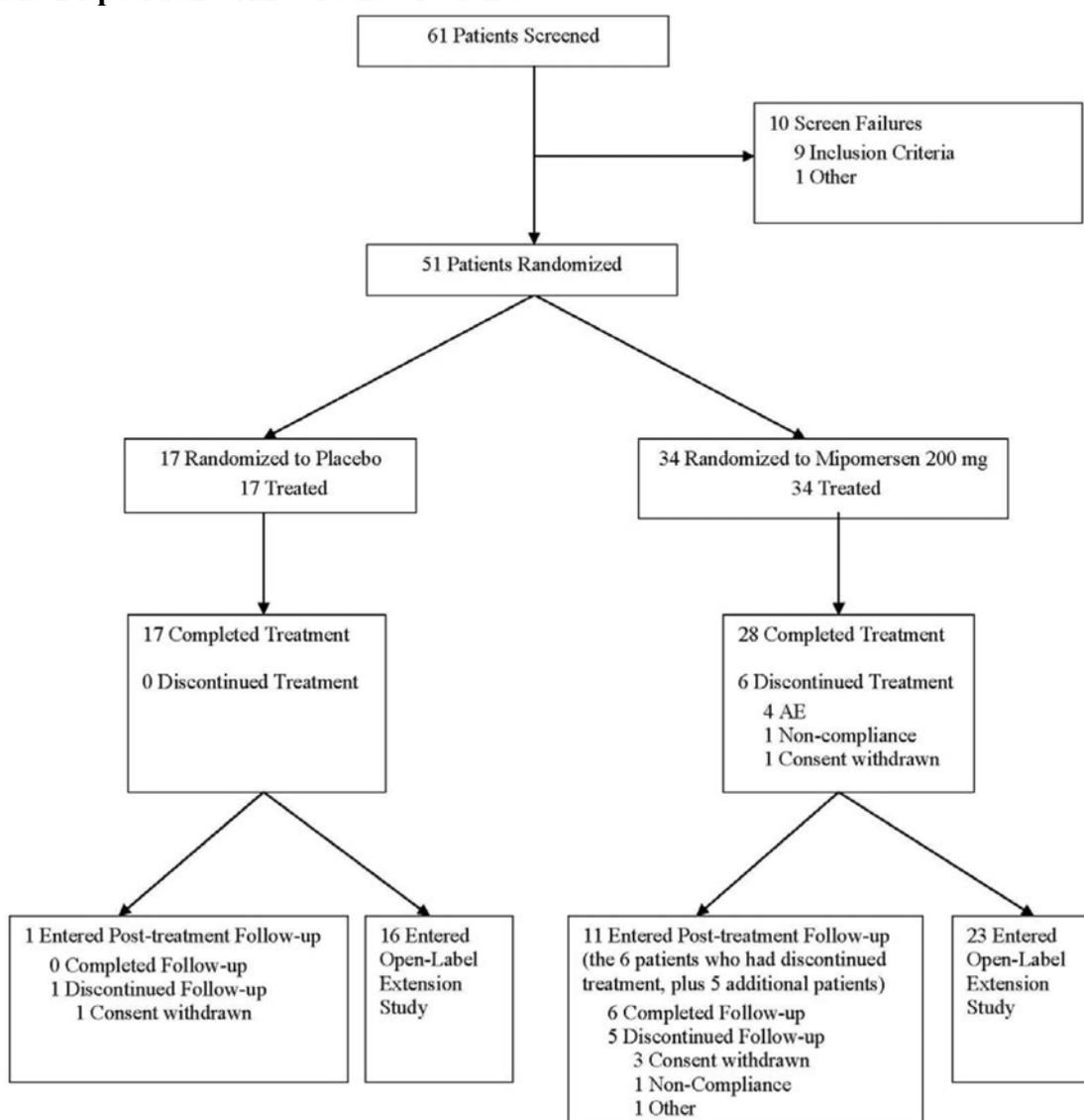
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Table below summarizes demographic and baseline characteristics for the Safety Set. Of the 51 patients, 29 (56.9%) were female. The majority of patients were White (38 [74.5%]). The median age was 27 years for patients in the mipomersen group and 38 years for patients in the placebo group. Seven (20.6%) patients in the mipomersen group and 3 (17.6%) patients in the placebo group were tobacco users. Fourteen (41.2%) patients in the mipomersen group and 6 (35.3%) patients in the placebo group were current users of alcohol.

Mean baseline fasting serum insulin levels and fasting HbA1c levels were similar for the treatment groups.

Seven (20.6%) patients in the mipomersen group and 1 (5.9%) patient in the placebo group had metabolic syndrome at baseline (for the definition of metabolic syndrome, see [Section 9.7.1.3](#)). With the exception of baseline metabolic syndrome, the treatment groups were comparable with respect to demographics and baseline characteristics.

Diagram for Patient Disposition – All Screened Patients



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!

Demographics and Baseline Characteristics – Safety Set

Parameter Statistic	Placebo (N = 17)	Mipomersen (N = 34)	P-Value
Age (year)			0.469
Mean (SD)	33.0 (14.1)	30.4 (11.5)	
Median (Q1, Q3)	38 (22, 45)	27 (22, 38)	
Min, Max	12, 53	14, 53	
Gender, n (%)			1.000
Male	7 (41.2)	15 (44.1)	
Female	10 (58.8)	19 (55.9)	
Ethnicity, n (%)			0.650
Hispanic or Latino	1 (5.9)	5 (14.7)	
Not Hispanic or Latino	16 (94.1)	29 (85.3)	
Race, n (%)			1.000
White	13 (76.5)	25 (73.5)	
Asian	3 (17.6)	8 (23.5)	
Black	1 (5.9)	1 (2.9)	
BMI (kg/m ²)			0.736
Mean (SD)	26.32 (4.41)	25.97 (5.81)	
Median (Q1, Q3)	26.4 (23.1, 29.6)	25.4 (21.6, 29.5)	
Min, Max	19.6, 35.0	16.6, 36.7	
Waist/hip ratio			0.368
Mean (SD)	0.83 (0.07)	0.85 (0.06)	
Median (Q1, Q3)	0.8 (0.8, 0.9)	0.9 (0.8, 0.9)	
Min, Max	0.7, 0.9	0.7, 0.9	
Metabolic syndrome, n (%) ^a			0.242
No	16 (94.1)	27 (79.4)	
Yes	1 (5.9)	7 (20.6)	
Tobacco use, n (%)			0.821
Current	3 (17.6)	7 (20.6)	
Non-current	3 (17.6)	4 (11.8)	
Never	11 (64.7)	23 (67.6)	
Alcohol use, n (%)			0.709
Current	6 (35.3)	14 (41.2)	
Non-current	3 (17.6)	3 (8.8)	
Never	8 (47.1)	17 (50.0)	
Fasting serum insulin (μIU/mL)			0.889
Mean (SD)	9.72 (5.98)	11.54 (14.78)	

Median (Q1, Q3)	6.9 (4.9, 14.0)	8.0 (5.1, 12.0)	
Min, Max	2.8, 20.5	2.3 87.4	
Fasting hemoglobin A1c (%)			
Mean (SD)	5.47 (0.22)	5.34 (0.37)	0.112
Median (Q1, Q3)	5.5 (5.3, 5.6)	5.3 (5.1, 5.5)	
Min, Max	5.1, 5.9	4.5, 6.1	

^aMetabolic syndrome is determined according to the American Heart Association and the National Heart, Lung, and Blood Institute definition.

BMI = body mass index; CI = confidence interval; Max = maximum; Min = minimum; Q1 = first quartile; Q3 = third quartile; SD = standard deviation.

Note: Wilcoxon rank-sum p-values are presented for continuous variables and Fisher's exact test p-values are presented for categorical variables.

Source: [TS-DM-PVAL-CS5.RTF](#)

Table above summarizes demographic and baseline characteristics for the Safety Set in ISIS 301012-CS5. Of the 51 patients, 29 (56.9%) were female. The majority of patients were White (38 [74.5%]). The median age was 27 years for patients in the mipomersen group and 38 years for patients in the placebo group. Seven (20.6%) patients in the mipomersen group and 3 (17.6%) patients in the placebo group were tobacco users. Fourteen (41.2%) patients in the mipomersen group and 6 (35.3%) patients in the placebo group were current users of alcohol.

Mean baseline fasting serum insulin levels and fasting HbA1c levels were similar for the treatment groups. Seven (20.6%) patients in the mipomersen group and 1 (5.9%) patient in the placebo group had metabolic syndrome at baseline.

The treatment groups were comparable with respect to demographics and baseline characteristics.

Statistical Methodologies

From the statistical Analysis Plan:

“STATISTICAL ANALYSIS

Generally, analysis will be per MCSAP (presented latter) but specifically for CS5 the clinical study protocol prescribes a two stage analysis.

Two Analysis Stages:

1. Treatment Period Analysis - Safety and efficacy for the *treatment period* (see MCSAP definitions [Section 2](#)) of the study will be analyzed once data cleaning is complete and the data are ready to be frozen and unblinded. This will consist of data accrual up to PET.

2. Post-treatment Assessment Analysis - Those patients who do not roll into an open-label extension study will have 24 weeks of post-treatment assessment, the first 2 of which will be covered in the Treatment Period Analysis. Therefore, the Post-treatment Assessment Analysis would cover 22 weeks of follow-up. These data will be presented separately from the Treatment Period Analysis.

However, based on the close proximity of the analysis plan finalization and last patient visit for the entire study, there will only be a single comprehensive analysis after the entire database has been locked.

...

6 SAMPLE SIZE, POWER AND RANDOMIZATION

Based upon prior clinical trial experience with mipomersen, it is estimated that the standard deviation of the percent change in LDL-C is approximately 22%. With 15 subjects in the control group and 30 subjects in the mipomersen-treated group, this study would have at least 80% power to detect a 20% difference between the two groups.

Mipomersen Core Statistical Analysis Plan (MCSAP)

Study Day -1 will be the day immediately preceding Study Day 1 and negative Study Days will be measured backward from Study Day -1. "Study Day x" and "Day x" will be used interchangeably in the MCSAP, the SSSAPs, and the analysis output.

2) Baseline

For efficacy assessment of lipid parameters, baseline will be defined as the average of the screening and Study Day 1 (pre-treatment) assessments. An assessment will not be included in this calculation if it is associated with a non-fasting blood draw or was drawn more than four weeks prior to Study Day 1. If the Study Day 1 and screening LDL-C values are more than 12% different (relative to the maximum value), then only Study Day 1 will be used because the Study Day 1 value represents the best estimate of the patient's condition at the beginning of study medication. This definition is consistent with the recommendation in "Guidelines for the Clinical Evaluation of Lipid-Altering Agents in Adults and Children", issued by the Food and Drug Administration (FDA) in September 1990. If it is determined that LDL-C can be averaged across pre-treatment visits for a patient according to the above algorithm, then all lipid parameters for that patient will be handled similarly.

For all other assessments, baseline will be defined as the assessments on Study Day 1, if available, or the next earlier assessment, if Study Day 1 is missing.

3) Primary efficacy timepoint (PET)

For efficacy parameters, the primary efficacy timepoint (PET) is the post-baseline visit closest to 14 days after the last dose of study treatment for which LDL-C is assessed. If two visits are equidistant to 14 days after the last dose of study treatment, the later will be designated the PET as this would be expected to provide the most conservative estimate of efficacy (i.e. lipid values would be expected to be rebounding toward baseline levels). In this way, the primary efficacy characterization for patients who discontinue early from study treatment, for whom efficacy determination is already compromised by reduced treatment exposure, is not additionally attenuated by using a lipid assessment far beyond the treatment experience (e.g. during the post-treatment assessment period) at which time the patient's lipid profile would be expected to rebound toward baseline.

For patients who discontinue treatment early, all efficacy assessments after their PET assessment will be considered part of the post-treatment assessment period and will not be included in the efficacy tabulations. Such lipid assessments are beyond the treatment period, which represents the efficacy assessment period for the patient, and, if included, would distort the description of efficacy over time because each timepoint would represent a combination of efficacy on treatment, for continuing patients, and efficacy at varying durations post-treatment, for those patients who had discontinued. Such data would have limited interpretability.

4) Study periods

All Phase 2 and 3 Mipomerson studies consist of 3 study periods: screening (pre- treatment), treatment, and post-treatment follow-up. Additional details pertaining to individual studies will be provided in the SSSAP.

The *screening* (pre-treatment) period is the time from a patient's informed consent up to the time of first dose. This period is generally within 4 weeks prior to the study treatment.

The *treatment period* spans the time during which the study treatment is administered until the later of the PET (refer to definition #3 above) or 14 days beyond the last study medication date.

The *post-treatment assessment period* starts on the day after the *treatment period* and ends on the day of the patient's last contact date within that study.

3.2 Full Analysis Set (FAS)

The FAS, which represents the practically-feasible intent-to-treat (ITT) population as delineated in ICH Guideline E9, will consist of the subset of the Safety Set with a valid baseline and at least one post-baseline LDL-C measure.

STATISTICAL ANALYSIS

Formal statistical inference (i.e. p-values) will be performed only for comparisons of the efficacy parameters between treatment groups unless otherwise specified.

Statistical significance will be concluded if $p \leq 0.05$.

Visit windows will be delineated in the SSSAPs.

All data captured in all studies will also be presented in patient level listings; all data collected during the post-treatment assessment period will be displayed only in data listings unless it represents the only post-baseline data for a parameter (e.g. the only post-treatment ECG data may arise at some point during the post-treatment assessment period) or tabulation is deemed informative (this will be delineated in the appropriate SSSAP).

Key data analyses will be provided by site for those sites with at least 20% of the randomized patients for the study.

Local laboratory assessments will only be included in listings.

Only central laboratory assessments with no indication of lack-of-validity (e.g. hemolyzed, not a fasting blood draw for lipids, etc.) will be used in efficacy analyses.

4.1.3 Demographics and Disease Characteristics and History

The following patient demographics and baseline background characteristics will be summarized, as available, by treatment group and overall for all three analysis sets defined in [Section 3](#):

1. Age, gender, race, ethnicity.
2. Use of tobacco (none, past, or current as reported on the CRF)
3. Use of alcohol (none, past, or current as reported on the CRF)
4. BMI
5. Waist/hip ratio.

4.2 Efficacy Analysis

4.2.1 Definitions of Efficacy Parameters

The primary efficacy parameter will be LDL-cholesterol.

Secondary efficacy parameters will be:

1. Apo B

2. Total cholesterol
3. Non-HDL-C

Tertiary efficacy parameters will include:

1. Triglycerides
2. Lp(a)
3. VLDL-C
4. LDL/HDL ratio
5. Apo A1
6. HDL-C (Note: the purpose of evaluating this parameter is to determine whether or not there is a clinically meaningful adverse trend)

Additional efficacy parameters may be defined as tertiary as appropriate in the SSSAPs.

4.2.2 Statistical Methods for Evaluation of Efficacy Parameters

The primary analysis of efficacy parameters will be assessment of the percent change from baseline to PET compared between treatment groups. Both the two-sample t-test and the Wilcoxon rank sum test will be assessed for the comparison between treatment groups. If the Kolmogorov-Smirnov test of normality is statistically significant ($p < 0.05$) then the Wilcoxon rank sum test results will be utilized. Otherwise, the two-sample t-test will be used. Changes within treatment groups will be assessed using the Wilcoxon signed rank test. Additional descriptive tabulations of categories of percent change from baseline to PET will be provided by treatment group (e.g. increase, 0-5% decrease, >5- 10% decrease, >10-15% decrease, >15-20% decrease, >20-25% decrease, >25-30% decrease, >30-35% decrease, >35-40% decrease, >40-45% decrease, >45-50% decrease, and >50% decrease).”

Reviewer’s Note: By this reviewer’s understanding, for test of normality, there should be two p-values – one for placebo responses and another for mipomersen responses. From the only one p-value (0.120), provided by the sponsor for the Kolmogorov-Smirnov test in the efficacy results Table, it appears that this p-value is for the test of equality of the two distributions (one for placebo responses and another for mipomersen responses)

Kolmogorov-Smirnov test may also be taken as a test of means or medians. However, the power is diluted by considering the differences in the distribution functions. Therefore, this reviewer is not concerned about the non-significance of the Kolmogorov-Smirnov test p-value, if anybody takes it as a test of equality of means or medians. Also, it is not a protocol mentioned or generally used test for testing the equality of means or medians.

“Inflation of type I error due to multiple secondary endpoints will be controlled by, first, the specification of a small number of secondary parameters and, second, use of sequential inferential approach in which statistical significance of the primary

parameter is required before drawing inferential conclusions about the first secondary parameter (refer to order of list in [Section 4.2.1](#)). Inferential conclusions about each successive parameter require statistical significance of the prior one. No further adjustments will be made for tertiary parameters.

Additionally, the results at each follow-up visit through Week 28, and the percent change from baseline to that follow-up visit, will be tabulated for all efficacy parameters. For analyses at each follow-up visit, if multiple values are recorded during a visit window, the average of all assessments will be used to provide the most robust estimate of a patient's lipid profile during that period of the study. The Wilcoxon rank sum test will be assessed for comparisons between treatment group and the Wilcoxon signed rank test will be used for assessment of changes within treatment groups.

Baseline lipid assessments will be compared between treatment groups using the Wilcoxon rank sum test. If a study has a difference in the number of patients between all randomized patients and the full analysis set, an additional tabulation of baseline lipid assessments among all randomized patients will be generated.

Sensitivity analyses of the primary efficacy parameter will consist of the following:

1. Percent change at the lipid assessment closest to 14 days after the last protocol-prescribed dosing day (i.e. in a 26 week treatment study, this would correspond to the Week 28 assessment). For patients completing 26 weeks of study treatment, this data will be identical to that in the PET analysis. However, for patients who discontinue study treatment early, this data may be substantially after their last dose of study medication. These data will be analyzed in the same way as PET data (see above) with the exception that no tabulations by site or by category of change will be provided.
2. Linear regression analyses and corresponding sub-group tabulations for the following factors: baseline LDL-C, age, sex, and race (e.g. white vs. non-white if supported by adequate distribution of patients). The linear regression analyses will consist of two models for each factor; the first model will include terms for treatment, factor, and treatment-by-factor interaction while the second model will only have terms for treatment and factor. These analyses will not be executed for phase 2 studies because the studies have sample sizes of approximately 8 patients per treatment group so such sub-group analyses could not be reliably interpreted.
3. Robustness of overall findings will be assessed by a qualitative comparison to LDL-C percent change from Day 1 to PET (i.e. only a single assessment will be used in the baseline determination)."

Changes in the Conduct of the Study or Planned Analyses

Amendments to the Protocol

There were 3 amendments to the original protocol, dated 18 July 2005. No patients were enrolled into the study under the original protocol or Amendment 1. The text presented in this study report incorporated the changes provided in the amendments:

Amendment 1 (12 January 2007)

(b) (4)

(b) (4)

Other modifications were made to incorporate knowledge gained from consultants and from ongoing clinical studies with mipomersen, as well as to maintain consistency with other ongoing mipomersen clinical studies. Minor changes were also made to improve the overall clarity of the original protocol. This amendment was finalized prior to study initiation.

□ Amendment 2 (10 May 2007)

(b) (4)

(b) (4)

Other modifications were made to incorporate knowledge gained from consultants and from ongoing clinical studies of mipomersen, as well as to maintain consistency with other ongoing mipomersen clinical studies. Minor changes were also made to improve the overall clarity of the original protocol. This amendment was finalized prior to study initiation.

□ Amendment 3 to the protocol (17 July 2008) disclosed a change of Sponsor (formerly Isis Pharmaceuticals, Inc.) to Genzyme Corporation. The safety reporting information was also updated to provide contact information for the Genzyme Pharmacovigilance Department. (b) (4)

The complete amendments are provided in Appendix 16.1.1 of the sponsor submission.

Changes to the Planned Analyses

The SAP contained the following changes in the conduct of the study or planned analyses:

- The protocol defined an All-Patients-Treated Population (all randomized patients who received at least 1 dose of study drug), a Per-Protocol Population (all randomized patients who received at least 13 doses of study drug and had no major protocol violations), and a Safety Population (all randomized patients who received at least 1 dose of study drug). These were replaced in the SAP with the Full Analysis Set, Per-Protocol Set, and the Safety Set.
- The protocol defined the cardiovascular risk markers hsCRP and LDL subclasses and particle size as efficacy parameters, whereas the SAP defined these as safety parameters. For this report, lipoprotein subclasses and particle size are presented with the efficacy results ([Section 11](#)) and hsCRP is presented with the safety results ([Section 12](#)).
- In addition to the efficacy parameters outlined in the protocol, the SAP included LDL/HDL ratio as an efficacy parameter.
- The SAP added the classification of patients with metabolic syndrome.
- The protocol defined baseline as the single observation immediately prior to first dose. In the SAP, baseline for efficacy assessments was defined as the average of the pre-dose assessments, which is aligned with the recommendation in Guidelines for the Clinical Evaluation of Lipid-Altering Agents in Adults and Children. The Phase 2 studies were also handled in this manner so this approach provided consistency.
- Consistent with the long half-life of mipomersen (approximately 5 weeks), the protocol included 26 weeks of treatment followed by 24 weeks of follow-up. The lipid profile continued to be evaluated during the safety follow-up period, but lipid measurements obtained during this period were not used as efficacy data. The protocol used a last observation carried forward approach in which the last observation after baseline but at or before Week 28 was to be used as the efficacy endpoint value. This approach could have resulted in a situation in which a patient who discontinued treatment 26 weeks could have had lipid measurements well beyond the last dose of study drug. The SAP defined the PET as the post-baseline visit closest to 14 days after the last dose of study drug. These 2 approaches would have yielded the same results unless a patient discontinued treatment substantially early and had follow-up, long-term lipid assessments accrued during the post-treatment assessment period. In such cases, the PET analysis provided a consistent concept of efficacy for all patients (i.e., efficacy realized 2 weeks after the last dose) while still being reasonably conservative because such patients would not have had the full opportunity to realize the efficacy potential of mipomersen. Therefore, the PET-based analysis yielded a

more scientifically interpretable characterization of efficacy.

The SAP states that summary tables will be presented for urinalysis parameters; however, because urinalysis is a mix of categorical and numerical data, summary tables are not provided.

The protocol and the SAP state that urine samples for drug compliance were to be collected and analyzed to verify self administration of study drug. Urine samples were collected, but not analyzed since there was no indication of dosing non-compliance.

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Results and Conclusions

Data Sets Analyzed

Table below presents a summary of the analysis sets. The Safety Set included 34 patients in the mipomersen group and 17 patients in the placebo group. The Full Analysis Set included the same 51 patients as in the Safety Set.

It was decided at the Blinded Evaluability Review Meeting that patients with a difference of more than 12% between screening and Day 1 LDL would be retained in the Per-Protocol Set because:

- x Of the 15 patients with more than 12% difference, roughly half of them had increases indicating that there was no trend in changes during the screening period, so the likelihood of this factor leading to a bias in the estimate of treatment effect is very low; and

- x The 12% variability derived from an FDA guidance (“Guidelines for the Clinical Evaluation of Lipid-Altering Agents in Adults and Children”, issued by the FDA in September 1990) is based on a large body of data on the variability of LDL-C in polygenic hypercholesterolemic patients, in whom clearance of LDL-C occurs predominantly via uptake by the LDL receptor, while variability may be higher among HoFH patients because of the greater contribution of non-receptor mediated uptake (i.e., residual receptor activity and the reticuloendothelial system).

It was decided at the Blinded Evaluability Review Meeting that if a patient’s compliance was <80% during participation in the treatment period, the patient was excluded from the Per-Protocol Set. Furthermore, because the historic PK profile for mipomersen shows that approximately 80% of steady-state tissue levels of mipomersen are expected after 12 weeks of treatment, it was decided at the Blinded Evaluability Review Meeting that a cutoff of strictly less than 12 weeks on study treatment would be used for excluding patients from the Per-Protocol Set.

Six patients were excluded from the Per-Protocol Set due to protocol deviations:

- x Patient 1501-8057 in the mipomersen group and Patient 1501-8285 in the placebo group were excluded because they started thyroid hormone replacement therapy during the study for hypothyroidism which was present at screening.
- x Patient 1501-8413 and Patient 1525-8169 both in the mipomersen group were excluded due to inadequate duration of treatment (8.1 weeks and 3.1 weeks, respectively).

- x Patient 1523-8309 in the mipomersen group was excluded due to low study drug compliance (65% compliant).
- x Patient 1525-8017 in the mipomersen group was excluded for failing to have a homozygous diagnosis.

Analysis Set Evaluability – All Patients Randomized

	Placebo (N = 17) n (%)	Mipomersen (N = 34) n (%)	Total (N = 51) n (%)
Number of patients randomized	17 (100.0)	34 (100.0)	51 (100.0)
Number of patients in the Safety Set	17 (100.0)	34 (100.0)	51 (100.0)
Number of patients in the Full Analysis Set	17 (100.0)	34 (100.0)	51 (100.0)
Number of patients in the Per-Protocol Set	16 (94.1)	29 (85.3)	45 (88.2)
Number of patients excluded:	1 (5.9)	5 (14.7)	6 (11.8)
Proscribed medication /changes	1 (5.9)	1 (2.9)	2 (3.9)
Low study drug compliance	0 (0.0)	1 (2.9)	1 (2.0)
Inadequate time on study drug	0 (0.0)	2 (5.9)	2 (3.9)
Inclusion/exclusion criteria violations	0 (0.0)	1 (2.9)	1 (2.0)

Source: [Table 14.1.2.3](#)

Tables below present the results for percent change in LDL-C from baseline to the PET for the Full Analysis Set (baseline and PET levels in gravimetric and International System [SI] units, respectively). The mean percent change in LDL-C was -24.7% for patients in the mipomersen group and -3.3% for patients in the placebo group. The treatment difference was statistically significant ($p < 0.001$).

For the mipomersen group, the mean LDL-C level was 438.9 mg/dL (11.37 mmol/L) at baseline and 326.2 mg/dL (8.45 mmol/L) at the PET; the mean absolute change in LDL-C was -112.7 mg/dL (-2.92 mmol/L). For the placebo group, the mean LDL-C level was 400.2 mg/dL (10.37 mmol/L) at baseline and 388.2 mg/dL (10.06 mmol/L) at the PET; the mean absolute change in LDL-C was -12.0 mg/dL (-0.31 mmol/L).

Percent Change in LDL Cholesterol From Baseline to the Primary
Efficacy Time Point (Gravimetric Units) – Full Analysis Set

Time point Statistic	Placebo (N = 17)	Mipomersen (N = 34)
Baseline (mmol/L)		
n	17	34
Mean (SD)	10.37 (3.666)	11.37 (3.588)
Min, Max	4.45, 16.54	4.92, 18.23
PET (mmol/L)		
n	17	34
Mean (SD)	10.06 (3.899)	8.45 (3.142)
Min, Max	3.34, 15.70	1.61, 15.20
Percent change		
n	17	34
Mean (SD)	-3.31 (17.06)	-24.65 (19.86)
Min, Max	-33.4, 43.1	-81.8, 2.1
95% CI	(-12.1, 5.5)	(-31.6,-17.7)
Wilcoxon signed rank test (p-value)	0.323	<0.001
t-test (p-value)		<0.001

For patients with TG <400 mg/dL, LDL-C was obtained using Friedewald's calculation; and for patients with TG ≥400 mg/dL, LDL-C was directly measured by the central laboratory using ultracentrifugation.

CI = confidence interval; Max = maximum; Min = minimum; PET = primary efficacy time point;

SD = standard deviation.

Percent Change in LDL Cholesterol From Baseline to the Primary Efficacy Time Point (SI Units) – Full Analysis Set

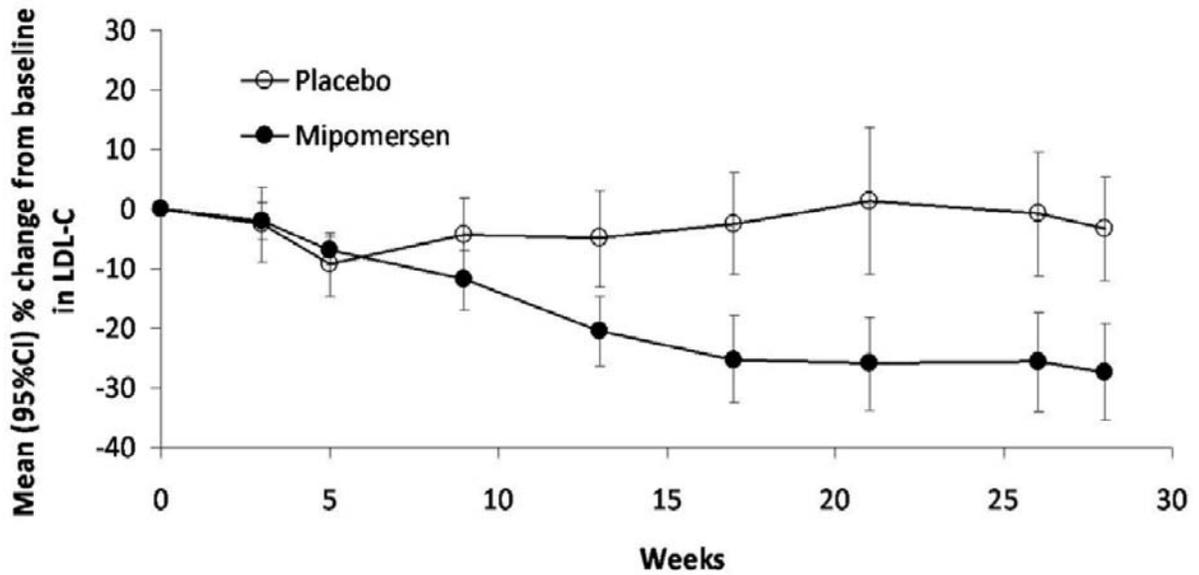
Time point Statistic	Placebo (N = 17)	Mipomersen (N = 34)
Baseline (mmol/L)		
n	17	34
Mean (SD)	10.37 (3.666)	11.37 (3.588)
Min, Max	4.45, 16.54	4.92, 18.23
PET (mmol/L)		
n	17	34
Mean (SD)	10.06 (3.899)	8.45 (3.142)
Min, Max	3.34, 15.70	1.61, 15.20
Percent change		
n	17	34
Mean (SD)	-3.31 (17.06)	-24.65 (19.86)
Min, Max	-33.4, 43.1	-81.8, 2.1
95% CI	(-12.1, 5.5)	(-31.6,-17.7)
Wilcoxon signed rank test (p-value)	0.323	<0.001
t-test (p-value)		<0.001

For patients with TG <400 mg/dL, LDL-C was obtained using Friedewald's calculation; and for patients with TG ≥400 mg/dL, LDL-C was directly measured by the central laboratory using ultracentrifugation.

CI = confidence interval; Max = maximum; Min = minimum; PET = primary efficacy time point;

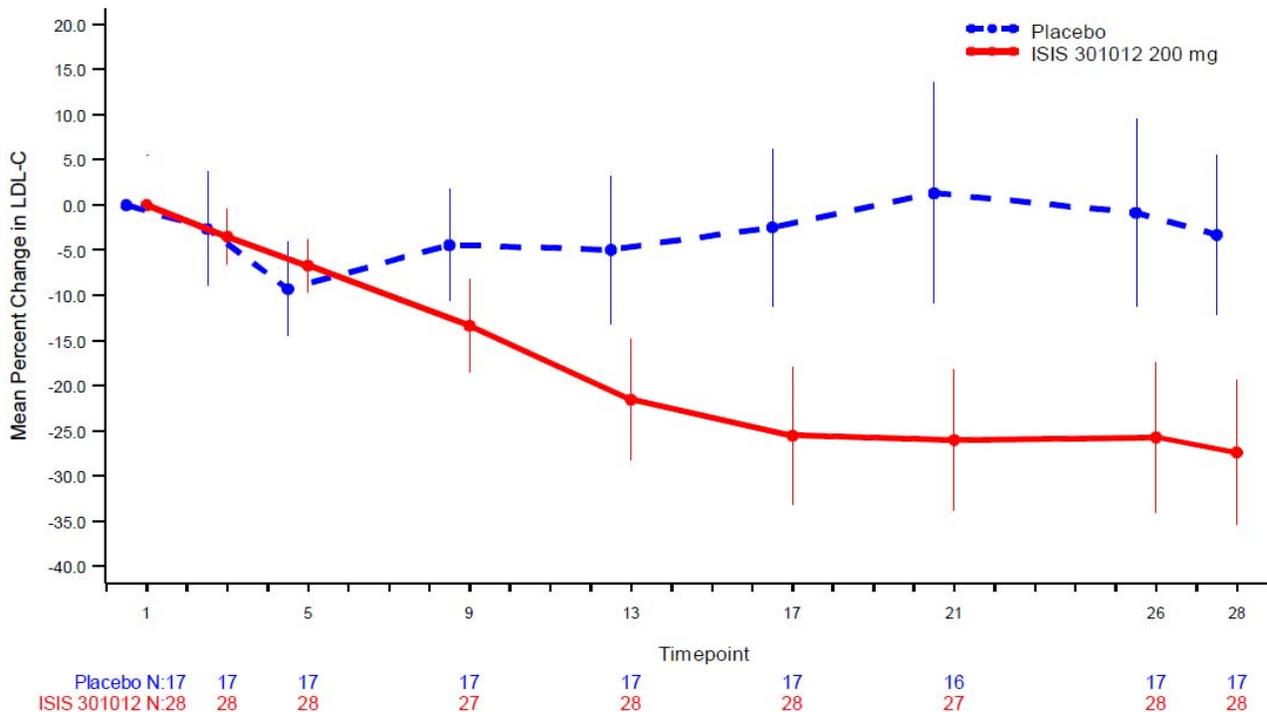
SD = standard deviation.

Source: [Table 14.2.1.1b](#)



Error bars indicate the 95% confidence intervals.
 LDL-C = low-density lipoprotein cholesterol.
 Source: [Table 14.2.5.1a](#)

Mean Percent Change in LDL-Cholesterol Over Time – Full Analysis Set, Patients Who Completed Study Treatment in ISIS 301012-CS5



Vertical bars indicate the 95% confidence intervals.
 ISIS 301012 = mipomersen; LDL-C = low-density lipoprotein cholesterol.
 Source: [ISIS 301012-CS5 CSR Addendum 1 Figure 6-1](#) and [Statistical Figure 14.2.2.1](#)

For patients in the Full Analysis Set who completed study treatment in ISIS 301012-CS5, a progressive decrease in LDL-C levels was observed in the mipomersen group compared with placebo during the first 16 weeks of treatment. From Week 17 to Week 28, the LDL-C levels remained generally stable.

§

Percent Change in LDL Cholesterol: Cumulative Distribution Function

Table below summarizes the numbers and percentages of patients in lipid response categories for the Full Analysis Set. Overall, approximately 80% of patients in the mipomersen group had at least a 10% decrease in lipid levels from baseline to PET compared with only 35% of patients in the placebo group. Seven (20.6%) patients in the mipomersen group and 2 (11.8%) patients in the placebo group had a 10% to 15% decrease in lipid levels from baseline to PET. Four (11.8%) patients in the mipomersen group had a >50% decrease in lipid levels from baseline to PET; no patients in the placebo group had a >50% decrease.

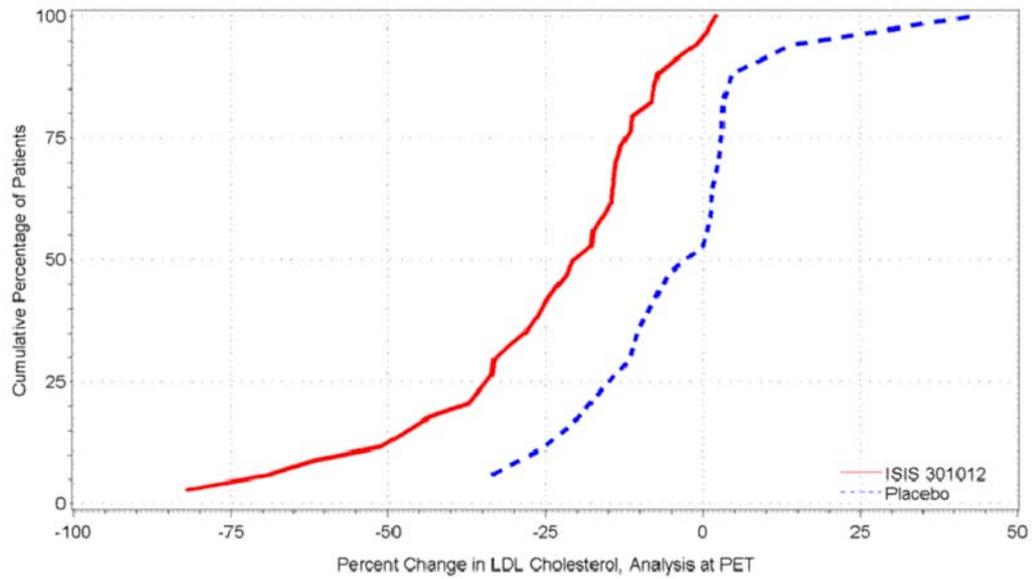
Categories of Lipid Response (Percent Change From Baseline to the Primary Efficacy Time Point) – Full Analysis Set

Categorical response	Placebo (N = 17)	Mipomersen (N = 34)
Increase	8 (47.1)	2 (5.9)
0% to 5% decrease	1 (5.9)	2 (5.9)
>5% to 10% decrease	2 (11.8)	3 (8.8)
>10% to 15% decrease	2 (11.8)	7 (20.6)
>15% to 20% decrease	2 (11.8)	3 (8.8)
>20% to 25% decrease	1 (5.9)	3 (8.8)
>25% to 30% decrease	0 (0.0)	3 (8.8)
>30% to 35% decrease	1 (5.9)	3 (8.8)
>35% to 40% decrease	0 (0.0)	2 (5.9)
>40% to 45% decrease	0 (0.0)	1 (2.9)
>45% to 50% decrease	0 (0.0)	1 (2.9)
>50% decrease	0 (0.0)	4 (11.8)

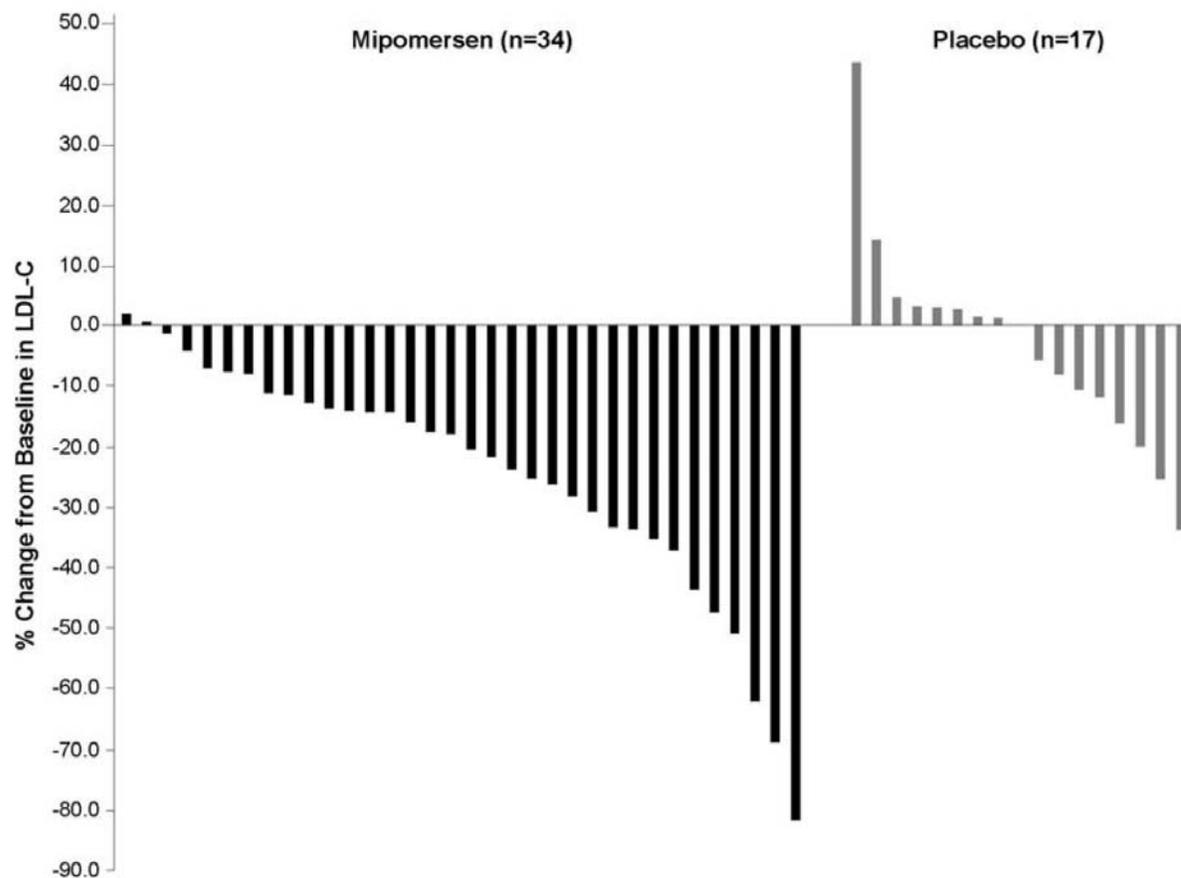
Source: [Table 14.2.3.1](#)

Figure below shows the cumulative distribution of individual patient percent change in LDL-C. This figure further illustrates that, across the range of LDL-C responses, higher percentages of patients in the mipomersen group had greater LDL-C reduction at the PET compared to the placebo group.

Figure for Cumulative Distribution Function for Change in HbA1c (pct) at week 26 (LOCF), Trial NN2211-1797 ITT Analysis Set



LDL = low-density lipoprotein; PET = primary efficacy time point.
Source: [Figure 14.2.1.1a](#)



LDL-C = low-density lipoprotein cholesterol.

Alternative Analyses

Regression Analysis of LDL-C at Primary Efficacy Timepoint (PET) Full Analysis Set

Model	Model Parameters	Parameter Estimate
Model 1 - Full Model	Intercept	-6.011
	Treatment group (Referent: Placebo)	-8.109
	Baseline LDL-C	0.261
	Treatment*baseline LDL-C interaction	-1.188
Model 2 - Reduced Model	Intercept	2.165

Treatment group (Referent: Placebo)	-20.823
Baseline LDL-C	-0.528

Regression Analysis of LDL-C at Primary Efficacy Timepoint (PET) Full Analysis Set

Model	Model Parameters	Parameter Estimate
Model 1 - Full Model	Intercept	15.153
	Treatment group (Referent: Placebo)	-45.133
	Age	-0.559
	Treatment*age interaction	0.734
Model 2 - Reduced Model	Intercept	1.090
	Treatment group (Referent: Placebo)	-21.697
	Age	-0.133

Regression Analysis of LDL-C at Primary Efficacy Timepoint (PET) Full Analysis Set

Model	Model Parameters	Parameter Estimate
Model 1 - Full Model	Intercept	-0.495
	Treatment group (Referent: Placebo)	-24.337
	Gender (Referent: Male)	-4.784
	Treatment*gender interaction	5.090
Model 2 - Reduced Model	Intercept	-2.503
	Treatment group (Referent: Placebo)	-21.392
	Gender (Referent: Male)	-1.371

Regression Analysis of LDL-C at Primary Efficacy
Timepoint (PET) Full Analysis Set

Model	Model Parameters	Parameter Estimate
Model 1 - Full Model	Intercept	-0.213
	Treatment group (Referent: Placebo)	-23.982
	Race (Referent: White)	-13.157
	Treatment*race interaction	11.574
Model 2 - Reduced Model	Intercept	-2.113
	Treatment group (Referent: Placebo)	-21.053
	Race (Referent: White)	-5.083

Regression Analysis of LDL-C at Primary Efficacy Timepoint
(PET) Per Protocol Set

Model	Model Parameters	Parameter Estimate
Model 1 - Full Model	Intercept	-6.067
	Treatment group (Referent: Placebo)	-4.581
	Baseline LDL-C	0.319
	Treatment*baseline LDL-C interaction	-1.400
Model 2 - Reduced Model	Intercept	2.640
	Treatment group (Referent: Placebo)	-19.595
	Baseline LDL-C	-0.527

A mixed model for repeated measures (MMRM) analysis was conducted using a restricted maximum likelihood-based approach. Analyses included the fixed, categorical effects of treatment, visit, and treatment-by-visit interaction, as well as the continuous, fixed covariate of baseline LDL-C.

An unstructured covariance structure was used to model the within-patient errors. The Satterthwaite approximation was used to estimate denominator degrees of freedom. The mean percent change at Week 28 for each treatment group was estimated. In addition, the mean difference (active – placebo), 95% confidence interval of the mean difference, and two-sided p-value at Week 28 were summarized using least-squares means.

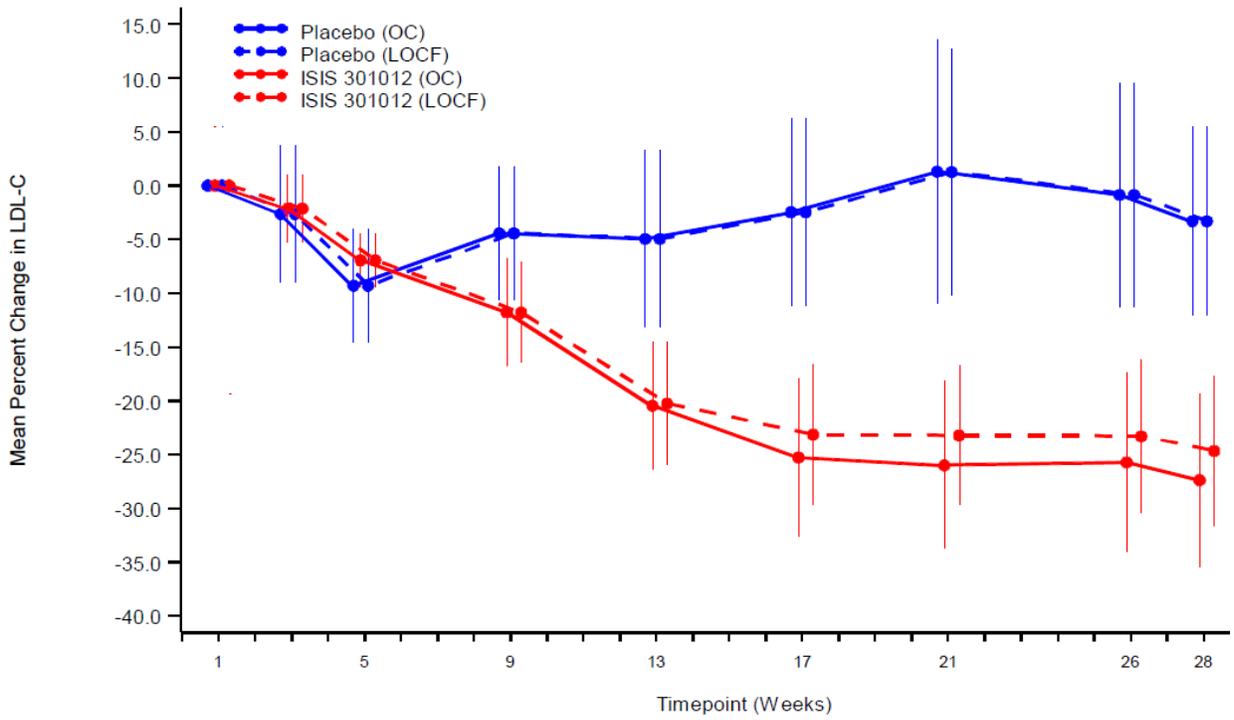
In these analyses, treatment with mipomersen resulted in a decline of 22% in LDL-C. Statistically significant ($p < 0.001$) and clinically meaningful differences compared with placebo were observed.

Mixed Model for Repeated Measures of LDL-C, Full Analysis Set for ISIS 301012-CS5

Study ID	Mean % Change (SE) at Week 28, Active	Mean % Change (SE) at Week 28, Placebo	Mean Difference (SE) in % Change, Active - Placebo	95% CI for Mean Difference in % Change, Active - Placebo	P-value
ISIS 301012-CS5	-25.48 (3.42)	-3.29 (4.65)	-22.19 (5.77)	(-33.81, -10.57)	<0.001

To further explore the effect of dropouts on the primary efficacy endpoint, a plot was created showing the mean (and 95% confidence interval) percent change in LDL-C over time. Each plot contains 4 lines: the observed cases (OC) for mipomersen and placebo groups and the last observation carried forward (LOCF) approach for mipomersen and placebo groups. Overall, these plots demonstrate that a progressive decrease in LDL-C levels was observed in the mipomersen group compared with placebo using both OC and LOCF.

Mean (95% CI) Percent Change in LDL-C over Time, Full Analysis Set in Study ISIS 301012-CS5



Placebo (OC) N:	17	17	17	17	17	17	16	17	17
Placebo (LOCF) N:	17	17	17	17	17	17	17	17	17
ISIS 301012 (OC) N:	34	34	34	32	33	29	27	28	28
ISIS 301012 (LOCF) N:	34	34	34	34	34	34	34	34	34

**Secondary Efficacy Results in Pivotal Study ISIS 301012-CS5
(Gravimetric Units) – Full Analysis Set**

Parameter Time Point	Treatment Arm		p-value
	Placebo (N=17) Mean (SD)	Mipomersen (N=34) Mean (SD)	
Apolipoprotein B-100 (mg/dL)			
Baseline	259.2 (84.4)	283.1 (78.4)	< 0.001
PET	252.6 (85.0)	205.4 (70.0)	
% Change from Baseline to PET	-2.5 (12.56)	-26.8 (17.04)	
95% CI	(-9.0, 3.9)	(-32.7, -20.8)	--
Total Cholesterol (mg/dL)			
Baseline	460.5 (132.0)	502.4 (144.5)	< 0.001
PET	452.1 (144.6)	389.7 (125.3)	
% Change from Baseline to PET	-1.98 (14.82)	-21.20 (17.69)	
95% CI	(-9.6, 5.6)	(-27.4, -15.0)	--
Non-High-Density Lipoprotein Cholesterol (mg/dL)			
Baseline	418.9 (144.5)	464.3 (145.4)	< 0.001
PET	409.1 (156.6)	345.8 (126.6)	
% Change from Baseline to PET	-2.90 (16.32)	-24.50 (19.17)	
95% CI	(-11.3, 5.5)	(-31.2, -17.8)	--

CI, confidence interval; PET, primary efficacy time point; SD, standard deviation

p-values from 2-sample t-test

95% CI is for the percent change from baseline to PET

Source: 2.7.3 Table 14

**Secondary Efficacy Results in Pivotal Study ISIS 301012-CS5 (SI Units) –
Full Analysis Set**

Parameter Time Point	Treatment Arm		p-value
	Placebo (N=17) Mean (SD)	Mipomersen (N=34) Mean (SD)	
Apolipoprotein B-100 (g/L)			
Baseline	2.59 (0.844)	2.83 (0.784)	<0.001
PET	2.53 (0.850)	2.05 (0.700)	
% Change from Baseline to PET	-2.5 (12.56)	-26.8 (17.04)	
95% CI	(-9.0, 3.9)	(-32.7, -20.8)	
Total Cholesterol (mmol/L)			
Baseline	11.93 (3.420)	13.01 (3.742)	<0.001
PET	11.71 (3.744)	10.09 (3.245)	
% Change from Baseline to PET	-2.0 (14.81)	-21.2 (17.69)	
95% CI	(-9.6, 5.6)	(-27.4, -15.0)	
Non-High-Density Lipoprotein Cholesterol (mmol/L)			
Baseline	10.85 (3.743)	12.03 (3.766)	<0.001
PET	10.60 (4.055)	8.96 (3.280)	
% Change from Baseline to PET	-2.90(16.30)	-24.5 (19.17)	
95% CI	(-11.3, 5.5)	(-31.2, -17.8)	

Results for more efficacy variables are in the next section.

Summary Results of All Studies as presented by the Sponsor

Some features of the whole Mipomersen development program and the four studies were presented before in Sections 1 and 2.

Cumulative Exposure to 200 mg/week Subcutaneous Mipomersen - All Patients and Homozygous Familial Hypercholesterolaemia Patients

Cumulative Exposure (Months) ^a	All Patients (N)	HoFH Patients (N)
≥6	243	41
Patient-years	282.2	48.2
≥12	113	25
Patient-years	211.4	38.0
≥18	75	7
Patient-years	166.8	16.5
≥24	54	6

Patient-years	128.8	14.5
---------------	-------	------

HoFH, homozygous familial hypercholesterolaemia

^a Cut-off for at least 6 months of dosing = 176 days, which represents 26 weeks of dosing. The cut-off for at least 12, 18, and 24 months is 358 days, 540 days, and 722 days, respectively.

Data are through 30 November

2011. Source: [ISS Statistical](#)

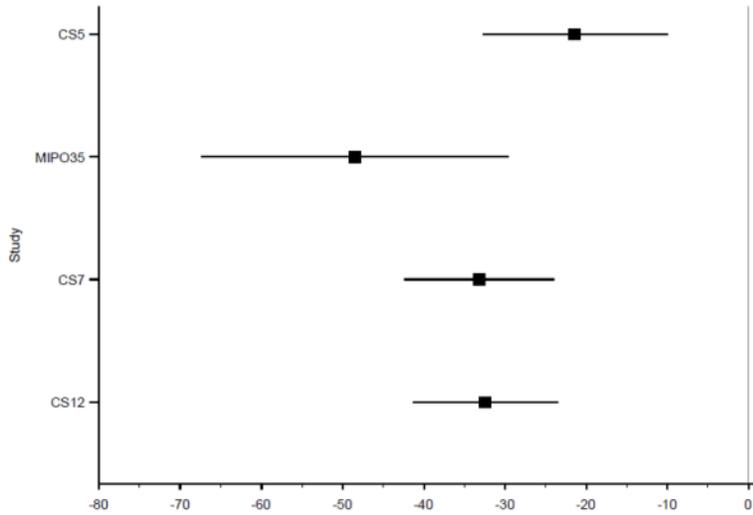
[Table 2](#)

Significance Levels for Changes in Lipid Parameters

Endpoint	Phase 3 Study			
	ISIS301012-CS5 (Pivotal)	MIPO3500108 (Supportive)	ISIS301012-CS7 (Supportive)	ISIS301012-CS12 (Supportive)
Primary Endpoint				
LDL-C	p<0.001	p<0.001	p<0.001	p<0.001
Secondary Endpoints				
Apo B-100	p<0.001	p<0.001	p<0.001	p<0.001
TC	p<0.001	p<0.001	p<0.001	p<0.001
Non-HDL-C	p<0.001	p<0.001	p<0.001	p<0.001
Tertiary Endpoints				
Lp(a)	p<0.01	p<0.001	p<0.001	p<0.001
VLDL-C	p<0.01	p<0.05	p<0.05	p<0.001
TG	p<0.05	p<0.05	p<0.05	p<0.001
LDL/HDL ratio	p<0.001	p<0.001	p<0.001	p<0.001

Source: [ISIS 301012-CS5 CSR Section 11.1](#); [MIPO3500108 CSR Section 11.1](#); [ISIS 301012-CS7 CSR Section 11.1](#); [ISIS 301012-CS12 CSR Section 11.1](#)

Low-Density Lipoprotein Cholesterol Percent Change from Baseline to Primary Efficacy Time Point Treatment Effects (Difference Between Mipomersen and Placebo Treatment) and 95% Confidence Intervals for Phase 3 Clinical Studies



Treatment Effect (mipomersen - placebo) in LDL Percent Change from Baseline to PET

CS5, Study ISIS 301012-CS5; CS7, Study ISIS 301012-CS7; CS12, Study ISIS 301012-CS12; LDL, low-density lipoprotein cholesterol; MIPO35, Study MIPO3500108; PET, primary efficacy time point

Summary of Efficacy Findings in Pivotal and Supportive Studies (Gravimetric Units) – Full Analysis Set

Parameter	ISIS 301012-CS5 ^a		MIPO3500108 ^b		ISIS 301012-CS7 ^b		ISIS 301012-CS12 ^b	
	Placebo (N=17)	Mipomersen (N=34)	Placebo (N=18)	Mipomersen (N=39)	Placebo (N=41)	Mipomersen (N=83)	Placebo (N=52)	Mipomersen (N=105)
LDL-C (mg/dL)	Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)	
Baseline	400.2 (141.5)	438.9 (138.6)	249.4 (84.3)	276.1 (72.1)	142.9 (51.6)	152.9 (48.7)	122.7 (38.6)	122.6 (31.7)
PET	388.2 (150.5)	326.2 (121.3)	263.9 (102.0)	174.9 (82.8)	146.4 (43.4)	103.9 (33.0)	113.3 (35.1)	75.3 (32.4)
% change from baseline	-3.3 (17.06)	-24.7 (19.85)*	12.5 (46.87)	-35.9 (24.71)*	5.2 (18.02)	-28.0 (26.99)*	-4.5 (24.22)	-36.9 (26.85)*
Apo B (mg/dL)	Mean (SD)		Mean (SD)		Mean (SD)		Median (Q1, Q3)	
Baseline	259.2 (84.4)	283.1 (78.4)	182.8 (48.6)	202.1 (49.1)	126.8 (33.2)	132.8 (33.9)	106 (98, 132)	114 (102, 129)
PET	252.6 (85.0)	205.4 (70.0)	193.7 (54.2)	126.8 (49.6)	133.8 (32.6)	95.0 (29.7)	108 (91, 122)	64 (52, 95)
% change from baseline	-2.5 (12.56)	-26.8 (17.04)*	11.4 (36.80)	-35.9 (22.95)*	7.0 (16.52)	-26.3 (22.16)*	-1.7 (-12.6, 7.5)	-40.6 (-53.0, -22.6)*
TC (mg/dL)	Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)	
Baseline	460.5 (132.0)	502.4 (144.5)	320.6 (87.2)	356.8 (77.0)	213.4 (54.6)	225.3 (51.5)	200.0 (42.1)	202.6 (36.8)
PET	452.1 (144.6)	389.7 (125.3)	341.5 (100.5)	251.5 (82.2)	219.0 (49.0)	176.0 (35.9)	192.2 (38.3)	147.4 (39.9)
% change from baseline	-2.0 (14.82)	-21.2 (17.69)*	11.1 (34.74)	-28.3 (20.43)*	3.9 (12.84)	-19.4 (19.25)*	-2.7 (14.58)	-26.4 (18.65)*
Non-HDL-C (mg/dL)	Mean (SD)		Mean (SD)		Mean (SD)		Median (Q1, Q3)	
Baseline	418.9 (144.5)	464.3 (145.4)	277.5 (88.3)	305.6 (78.3)	165.3 (54.5)	175.5 (51.1)	144 (125, 175)	144 (132, 171)
PET	409.1 (156.6)	345.8 (126.6)	296.7 (103.8)	198.1 (85.3)	168.2 (47.5)	125.2 (37.8)	140 (115, 165)	90 (67, 116)
% change from baseline	-2.9 (16.32)	-24.5 (19.17)*	14.2 (47.75)	-34.0 (23.80)*	3.7 (16.04)	-25.1 (25.71)*	-1.2 (-13.6, 11.5)	-38.7 (-54.0, -24.2)*
Parameter	ISIS 301012-CS5 ^a		MIPO3500108 ^b		ISIS 301012-CS7 ^b		ISIS 301012-CS12 ^b	
	Placebo (N=17)	Mipomersen (N=34)	Placebo (N=18)	Mipomersen (N=39)	Placebo (N=41)	Mipomersen (N=83)	Placebo (N=52)	Mipomersen (N=105)
Lp(a) (mg/dL)	Mean (SD)		Mean (SD)		Median (Q1, Q3)		Mean (SD)	
Baseline	66.3 (53.1)	64.3 (41.0)	32.4 (28.5)	61.3 (68.4)	53 (17, 108)	45 (13, 93)	51.1 (48.6)	54.3 (57.0)
PET	61.6 (52.6)	43.8 (32.1)	32.1 (28.1)	43.3 (54.3)	51 (18, 108)	35 (9, 56)	49.5 (47.3)	39.6 (47.0)
% change from baseline	-7.9 (21.87)	-31.1 (23.02)*	-1.5 (25.74)	-32.7 (32.98)*	0.0 (-8.0, 13.0)	-21.1 (-37.9, 0.0)*	2.3 (28.09)	-24.0 (24.47)*

Apo, B, apolipoprotein B; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein(s); PET, primary efficacy time point; Q1, first quartile; Q3, third quartile; SD, standard deviation, TC, total cholesterol

Data presented as means, with p-values calculated using the 2 sample t-test, unless the result of the Kolmogorov Smirnov test was ≤ 0.05 (indicating non-normal distribution, in which case data are presented as medians, with p-values calculated using the Wilcoxon rank-sum test.

*The percent changes from baseline in the mipomersen group was statistically significant ($p < 0.001$) for all 4 studies.

^a Pivotal study.

^b Supportive study.

Source: 2.7.3 Table 6

Studies ISIS 301012-CS5, MIPO3500108, ISIS 301012-CS7, and ISIS 301012-CS12 Analyses of Lipid and C-Reactive Protein (CRP) Percent Changes from Baseline to Primary Efficacy Timepoint (PET), Stratified by Study Full Analysis Set

Parameter	Mean % Change (SE) Active	Mean % Change (SE) Placebo	Mean Difference (SE) in % Change Active - Placebo	95% CI for Mean Difference in % Change Active - Placebo	Treatment Difference P-value
LDL Cholesterol, Analysis	-31.64 (2.55)	1.87 (3.04)	-33.52 (2.83)	(-39.08, -27.95)	<0.001
Apolipoprotein B	-31.67 (2.68)	2.52 (3.02)	-34.19 (2.38)	(-38.87, -29.50)	<0.001
Cholesterol, Total	-23.58 (1.79)	1.70 (2.16)	-25.28 (2.06)	(-29.33, -21.23)	<0.001
Non-HDL-C	-29.91 (2.49)	2.25 (2.93)	-32.16 (2.64)	(-37.35, -26.97)	<0.001
Triglycerides	-14.60 (3.78)	10.86 (4.53)	-25.46 (4.28)	(-33.88, -17.05)	<0.001
Lipoprotein (a)	-26.39 (2.71)	-0.87 (3.14)	-25.52 (2.71)	(-30.84, -20.19)	<0.001
VLDL Cholesterol, Analysis	-14.64 (3.75)	10.64 (4.52)	-25.28 (4.31)	(-33.75, -16.81)	<0.001
LDL/HDL Ratio	-33.68 (2.47)	-0.39 (3.13)	-33.29 (3.29)	(-39.75, -26.82)	<0.001
Apolipoprotein A1	-0.93 (2.59)	3.03 (2.72)	-3.96 (1.45)	(-6.81, -1.10)	0.007
HDL Cholesterol	6.59 (2.70)	4.90 (2.94)	1.68 (1.99)	(-2.23, 5.60)	0.398
Apo B/Apo A1 Ratio	-30.11 (2.45)	1.09 (2.87)	-31.20 (2.57)	(-36.25, -26.15)	<0.001
C-Reactive Protein	59.15 (12.25)	36.86 (17.15)	22.28 (21.08)	(-19.16, 63.73)	0.291

3.3 Evaluation of Safety

This reviewer has not performed any safety analysis.

3.4 Benefit:Risk Assessment (Optional)

4. FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

Note: Adjustments for multiplicity in so many subgroups cannot be properly done on a post hoc basis, when an adjustment method is not mentioned prospectively. Subgroup results should not be taken as confirmatory.

Results of the demographic characteristics (at baseline) and other prognostic factors were presented before. There were no significant imbalances between the treatment groups.

4.1 Gender, Race, Age, and Geographic Region

Results from Phase 3 Studies

Sponsor's Note: It is important to remember that the baseline LDL-C and age characteristics for ISIS 301012-CS5 patients were different than the other studies. These patients are younger and have higher baseline LDL-C values, both indicative of their disease. As mentioned in Sequence 0012, region was only summarized in the pooled analyses.

In the 4 Phase 3 studies, regression analyses were performed to assess the impact of the following demographic and baseline characteristics on the efficacy of mipomersen: age, gender, race (White or non-White), and baseline LDL-C value (Table below). Some interaction p-values are statistically significant.

Studies ISIS 301012-CS5, MIPO3500108, ISIS 301012-CS7, and ISIS 301012-CS12
Subgroup Analyses of Percent Change from Baseline in LDL-C Pooled Across Phase 3 Studies, Full Analysis Set

	Treatment x covariate interaction P-value	Mean % Change (SE) at PET, Active	Mean % Change (SE) at PET, Placebo	Mean Difference (SE) in % Change, Active - Placebo	95% CI for Mean Difference in % Change, Active - Placebo	Treatment Difference P-value
Gender	<0.001					
Male		-25.80 (2.91)	-2.09 (3.60)	-23.71 (3.74)	(-31.06, -16.36)	<0.001
Female		-38.06 (2.97)	6.99 (3.90)	-45.05 (4.14)	(-53.19, -36.92)	<0.001
Race	0.317					
White		-29.76 (2.48)	2.66 (3.08)	-32.42 (3.09)	(-38.49, -26.36)	<0.001
Non-White		-41.75 (4.47)	-1.79 (5.80)	-39.96 (6.87)	(-53.46, -26.46)	<0.001
Age (years)	0.134					
<Median(<55)		-27.42 (2.84)	1.45 (3.51)	-28.87 (3.98)	(-36.69, -21.05)	<0.001
≥Median(≥55)		-35.92 (2.81)	1.44 (3.82)	-37.36 (4.02)	(-45.27, -29.45)	<0.001
Baseline LDL-C (mg/dL)	0.428					
<Median(<144)		-21.91 (5.28)	12.78 (5.62)	-34.69 (3.82)	(-42.21, -27.18)	<0.001
≥Median(≥144)		-36.07 (4.94)	-5.76 (5.54)	-30.32 (3.97)	(-38.12, -22.51)	<0.001
Region	0.916					
North America and Western Europe		-33.64 (2.86)	-0.04 (3.41)	-33.60 (3.14)	(-39.77, -27.42)	<0.001
Other		-26.27 (4.52)	6.56 (5.84)	-32.83 (6.52)	(-45.66, -20.00)	<0.001

The following interaction p-values were ≤ 0.1 in the pooled analysis:

Gender (interaction $p < 0.001$) - the treatment effect in females was larger than that seen in males

Treatment-by-Factor p-Values in Individual Phase 3 Studies – Full Analysis Set

Factor	Phase 3 Study			
	ISIS 301012-CS5	MIPO3500108	ISIS 301012-CS7	ISIS 301012-
Age	0.099	0.249	0.959	0.027
Gender	0.664	0.001	0.051	0.045
Race	0.380	0.889	0.066	0.074
Baseline LDL-C	0.463	0.074	0.168	0.651

LDL-C, low-density lipoprotein cholesterol

Source: ISIS 301012-CS5 CSR Table 14.2.2.1; MIPO3500108 CSR Table 14.2.2.1; ISIS 301012-CS7 CSR Table 14.2.2.1; and ISIS 301012-CS12 CSR Table 14.2.2.1

The following interaction p-values were ≤ 0.1 in individual studies.

□ ISIS 301012-CS5:

o Age (interaction $p=0.056$) - the treatment effect in younger patients tended to be larger than that seen in older patients

□ ISIS 301012-CS7:

o Gender (interaction $p=0.051$) – the treatment effect in females tended to be larger than that seen in males

o Race (interaction $p=0.066$) – the treatment effect in Non-White patients tended to be larger than that seen in White patients, however there were only 2 mipomersen and 3 placebo patients in the Non-White group and both mipomersen patients had greater than 70% LDL-C reduction

□ ISIS 301012-CS12:

o Gender (interaction $p=0.045$) - the treatment effect in females tended to be larger than that seen in males

o Race (interaction $p=0.074$) - the treatment effect in Non-White patients tended to be larger than that seen in White patients

o Age (interaction $p=0.054$) - the treatment effect in older patients tended to be larger than that seen in younger patients

□ MIPO3500108:

o Gender (interaction $p=0.001$) - the treatment effect in females was larger than that seen in males

Despite indication of a treatment interaction, for a given covariate, the subgroups typically had overlapping treatment effect confidence intervals. The only 2 cases where the subgroup treatment effect confidence intervals did not overlap were for the gender effect in MIPO35 and the pooled analysis.

Due to small subgroup sample sizes in some of the individual studies (particularly CS5 and MIPO35), statistical significance between treatments within each subgroup was not always achieved.

Appendix Table 1 provides a summary of mean changes in low-density lipoprotein cholesterol (LDL-C) from baseline to the primary efficacy time point (PET) in the pivotal and supportive studies of mipomersen according to the prespecified subgroups of gender, race, age, and baseline LDL-C analysed in each clinical study report. Summaries by region were not conducted for the individual pivotal and supportive studies because the overall groups that were established were not always present in each study (e.g., study ISIS 301012-CS7 and study ISIS 301012-CS12 enrolled patients from North America only).

Table below provides a summary of mean changes in low-density lipoprotein cholesterol (LDL-C) from baseline to the primary efficacy time point (PET) in the pivotal and supportive studies of mipomersen according to the prespecified subgroups of gender, race, age, and baseline LDL-C analysed in each clinical study report. Summaries by region were not conducted for the individual pivotal and supportive studies because the overall groups that were established were not always present in each study (e.g., study ISIS 301012-CS7 and study ISIS 301012-CS12 enrolled patients from North America only).

Summary of Primary Efficacy Endpoint Results in Pivotal and Supportive Studies by Gender, Age, Race, and Baseline Low-Density Lipoprotein Cholesterol (Gravimetric Units) – Full Analysis Set

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Subgroup	ISIS 301012-CS5 ^a		MIPO3500108 ^b		ISIS 301012-CS7 ^b		ISIS 301012-CS12 ^b	
	Placebo (N=17)	Mipomersen (N=34)	Placebo (N=18)	Mipomersen (N=39)	Placebo (N=41)	Mipomersen (N=83)	Placebo (N=52)	Mipomersen (N=105)
	Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)	
Gender – Male								
n	7	15	7	18	28	50	29	51
Baseline	450.6 (89.8)	399.5 (155.6)	276.4 (84.2)	267.4 (82.0)	141.2 (57.5)	145.6 (35.4)	127.3 (42.1)	122.5 (35.9)
PET	444.0 (76.9)	297.1 (122.6)	234.9 (95.5)	193.6 (78.0)	144.4 (46.8)	111.2 (32.2)	111.8 (36.0)	80.1 (31.1)
% change from baseline	-0.49 (10.19)	-24.83 (20.86)	-14.66 (24.46)	-26.97 (20.41)	5.87 (20.92)	-19.97 (27.72)	-8.61 (26.63)	-32.73 (24.96)
p-value	0.813	<0.001	0.109	<0.001	0.168	<0.001	0.078	<0.001
Gender – Female								
n	10	19	11	21	13	32	21	50
Baseline	364.9 (163.9)	470.1 (118.5)	232.2 (83.7)	283.6 (63.5)	146.5 (37.7)	164.3 (63.3)	116.2 (33.2)	122.8 (27.0)
PET	349.2 (179.5)	349.2 (118.5)	282.5 (106.1)	159.0 (85.2)	150.7 (36.0)	92.4 (31.4)	115.4 (34.6)	70.4 (33.2)
% change from baseline	-5.28 (20.92)	-24.53 (19.60)	29.85 (50.28)	-43.60 (25.93)	3.67 (9.70)	-40.60 (20.52)	1.10 (19.68)	-41.21 (28.25)
p-value	0.250	<0.001	0.007	<0.001	0.127	<0.001	0.602	<0.001
Race – White								
n	13	24	15	33	38	80	38	79
Baseline	423.3 (144.6)	475.7 (123.6)	259.7 (86.5)	275.4 (77.4)	142.1 (52.2)	152.1 (48.0)	126.3 (41.8)	123.3 (34.4)
PET	419.5 (149.4)	351.5 (110.9)	273.8 (105.9)	176.0 (84.3)	146.7 (44.4)	105.3 (31.8)	115.7 (39.2)	79.5 (32.1)
% change from baseline	-0.21 (17.51)	-24.19 (19.54)	13.58 (51.07)	-35.53 (24.39)	5.90 (18.40)	-26.78 (26.11)	-5.27 (25.71)	-33.47 (26.32)
p-value	0.850	<0.001	0.454	<0.001	0.038	<0.001	0.349	<0.001
Race – Non-White								
n	4	10	3	6	3	2	12	22
Baseline	325.0 (115.4)	350.7 (138.1)	198.0 (57.5)	280.5 (33.4)	152.5 (50.9)	185.3 (90.2)	111.3 (23.9)	120.2 (19.4)
PET	286.5 (116.4)	265.6 (129.4)	214.7 (75.1)	169.0 (80.4)	143.3 (34.0)	45.5 (37.5)	105.8 (15.4)	60.3 (29.5)
% change from baseline	-13.37 (12.21)	-25.78 (21.63)	7.34 (19.18)	-38.10 (28.76)	-4.08 (9.65)	-77.72 (9.39)	-2.21 (19.54)	-49.32 (25.55)
p-value	0.250	0.002	0.750	0.031	1.000	0.500	0.733	<0.001
Age – Below the Median								
n	7	18	9	20	20	42	24	41
Baseline	401.9 (62.6)	451.7 (140.8)	244.7 (116.6)	281.8 (62.8)	144.6 (35.3)	161.2 (48.9)	128.3 (49.0)	125.4 (34.2)
PET	414.1 (95.9)	303.6 (108.8)	280.0 (128.7)	210.3 (86.9)	146.7 (31.2)	112.5 (33.6)	113.6 (40.9)	83.9 (33.1)
% change from baseline	3.36 (20.77)	-29.61 (20.80)	26.96 (59.69)	-25.59 (21.22)	4.35 (22.60)	-26.81 (24.88)	-7.36 (25.18)	-29.82 (30.29)
p-value	0.938	<0.001	0.250	<0.001	0.430	<0.001	0.314	<0.001
Age – Above the Median								
n	10	16	9	19	21	40	26	60
Baseline	399.0 (181.6)	424.6 (139.1)	254.1 (38.3)	270.2 (82.1)	141.3 (64.3)	144.3 (47.6)	117.5 (25.6)	120.7 (29.9)
PET	370.1 (182.4)	351.6 (132.9)	247.9 (70.4)	137.7 (60.3)	146.1 (53.3)	94.8 (30.2)	113.0 (29.6)	69.4 (30.8)
% change from baseline	-7.98 (13.07)	-19.09 (17.73)	-1.87 (25.25)	-46.80 (23.88)	5.95 (12.76)	-29.29 (29.31)	-1.93 (23.49)	-41.78 (23.25)
p-value	0.164	<0.001	0.910	<0.001	0.027	<0.001	0.720	<0.001
Baseline LDL-C – Below the Median								
n	10	15	10	18	24	37	26	51
Baseline	306.2 (91.4)	306.7 (65.7)	193.4 (49.6)	213.4 (35.9)	117.4 (15.9)	116.5 (17.4)	96.0 (12.7)	100.6 (13.0)
PET	306.4 (133.6)	253.5 (86.4)	220.9 (51.3)	138.9 (52.0)	129.2 (23.7)	92.6 (33.4)	98.5 (20.8)	68.0 (27.0)
% change from baseline	-1.75 (21.67)	-19.17 (18.99)	23.59 (57.23)	-33.88 (24.11)	10.51 (15.97)	-18.52 (31.80)	3.55 (22.65)	-31.34 (28.39)
p-value	0.820	<0.001	0.232	<0.001	0.002	<0.001	0.420	<0.001

	ISIS 301012-CS5 ^a		MIPO3500108 ^b		ISIS 301012-CS7 ^b		ISIS 301012-CS12 ^b	
	Placebo (N=17)	Mipomersen (N=34)	Placebo (N=18)	Mipomersen (N=39)	Placebo (N=41)	Mipomersen (N=83)	Placebo (N=52)	Mipomersen (N=105)
Baseline LDL-C – Above the Median								
n	7	19	8	21	17	45	24	50
Baseline	534.5 (71.5)	543.3 (75.9)	319.4 (63.4)	329.9 (46.9)	178.9 (62.7)	182.9 (45.7)	151.6 (36.3)	145.1 (29.1)
PET	505.1 (80.9)	383.6 (115.2)	317.8 (126.2)	205.8 (92.4)	170.7 (53.1)	113.1 (30.0)	129.3 (40.6)	82.7 (35.8)
% change from baseline	-5.54 (7.86)	-28.99 (19.93)	-1.27 (27.03)	-37.67 (25.68)	-2.36 (18.50)	-35.83 (19.37)	-13.29 (23.19)	-42.63 (24.14)
p-value	0.219	<0.001	0.844	<0.001	0.747	<0.001	0.012	<0.001

LDL-C, low-density lipoprotein cholesterol; PET, primary efficacy time point; SD, standard deviation

p-values from Wilcoxon signed-rank test

^a Pivotal study.

^b Supportive study.

Source: ISIS 301012-CS5 CSR Table 14.2.6.1.1a, Table 14.2.6.1.1b, Table 14.2.7.1.1a, Table 14.2.7.1.1b, Table 14.2.8.1.1a, Table 14.2.8.1.1b, Table 14.2.9.1.1a, and Table 14.2.9.1.1b; MIPO3500108 CSR Table 14.2.6.1.1a, Table 14.2.6.1.1b, Table 14.2.7.1.1a, Table 14.2.7.1.1b, Table 14.2.8.1.1a, Table 14.2.8.1.1b, Table 14.2.9.1.1a, and Table 14.2.9.1.1b; ISIS 301012-CS7 CSR Table 14.2.6.1.1a, Table 14.2.6.1.1b, Table 14.2.7.1.1a, Table 14.2.7.1.1b, Table 14.2.8.1.1a, Table 14.2.8.1.1b, Table 14.2.9.1.1a, and Table 14.2.9.1.1b; and ISIS 301012-CS12 CSR Table 14.2.6.1.1a, Table 14.2.6.1.1b, Table 14.2.7.1.1a, Table 14.2.7.1.1b, Table 14.2.8.1.1a, Table 14.2.8.1.1b, Table 14.2.9.1.1a, and Table 14.2.9.1.1b

In addition to the analyses presented by study in Table above, demographic and baseline characteristics were investigated to explore the treatment effect within subgroups when using the combined data from all four Phase 3 studies (pooled Phase 3 studies). Similar to the analyses presented by study, race was grouped into White and non-White patients, as the majority of patient were White. Age was grouped into decades of ≤ 19 , 20 to 29, 30 to 39, 40 to 49, 50 to 59, 60 to 69, and ≥ 70 years. Baseline LDL-C was group as ≤ 100 , >100 to 200, >200 to 300, >300 to 400, and >400 mg/dL. Geographic region was grouped as North America and Western Europe (United States, Canada, United Kingdom, and Germany), and Other (South Africa, Czech Republic, Brazil, Singapore, and Taiwan) in order to keep North America and Western Europe together due to having a consistent standard of care (as recommended by the FDA during the Special Protocol Assessment [SPA] for study MIPO3801011). There were no high-enrolling sites that made it reasonable to analyze by site.

Table below provides a summary of the subgroups used in the mixed model analysis of the pooled Phase 3 data.

Summary of Subgroups used in the Mixed Model Analysis of the Pooled Phase 3 Data – Full Analysis Set

Category Subgroup	Placebo (N=126) n (%)	Mipomersen (N=256) n (%)	Total (N=382) n (%)
Gender			
Male	71 (56.3)	134 (52.3)	205 (53.7)
Female	55 (43.7)	122 (47.7)	177 (46.3)
Race			
White	104 (82.5)	216 (84.4)	320 (83.8)
Non-White	22 (17.5)	40 (15.6)	62 (16.2)
Age (years)			
≤19	5 (4.0)	6 (2.3)	11 (2.9)
20 - 29	5 (4.0)	16 (6.3)	21 (5.5)
30 - 39	5 (4.0)	19 (7.4)	24 (6.3)
40 - 49	29 (23.0)	39 (15.2)	68 (17.8)
50 - 59	38 (30.2)	82 (32.0)	120 (31.4)
60 - 69	32 (25.4)	68 (26.6)	100 (26.2)
≥70	12 (9.5)	26 (10.2)	38 (9.9)
Baseline Low-Density Lipoprotein Cholesterol (mg/dL)			
≤100	17 (13.5)	21 (8.2)	38 (9.9)
>100 - 200	75 (59.5)	158 (61.7)	233 (61.0)
>200 - 300	16 (12.7)	29 (11.3)	45 (11.8)
>300 - 400	8 (6.3)	28 (10.9)	36 (9.4)
>400	10 (7.9)	20 (7.8)	30 (7.9)
Region			
North America and Western Europe	102 (81.0)	209 (81.6)	311 (81.4)
Other	24 (19.0)	47 (18.4)	71 (18.6)

Source: [TS-SUBGROUP-PDAP3.rtf](#)

For each demographic and baseline factor, a mixed model was run. Fixed effects included the covariate, treatment, and the treatment-by-covariate interaction and study was included as a random effect. The Satterthwaite approximation was used to estimate denominator degrees of freedom. The mean percent change at PET for each treatment group was estimated. In addition, the mean difference (active – placebo), 95% confidence interval of the mean difference, and two-sided p-value were summarized using least-squares means.

Table below provides the results of the mixed model analysis of the LDL-C results by subgroup in the pooled Phase 3 studies. Overall, the mean percent change in LDL-C from baseline to PET was consistent across the subgroup factors. Some subgroups had very small numbers of patients; therefore, these data should be considered in light of the small numbers of data points.

Subgroup Analyses of Percent Change from Baseline in Low-Density Lipoprotein Cholesterol Pooled Across Phase 3 Studies – Full Analysis Set

Category Subgroup	LS Mean % Change (SE) at PET, Active	LS Mean % Change (SE) at PET, Placebo	LS Mean Difference (SE) in % Change, Active - Placebo	95% CI for Mean Difference in % Change, Active - Placebo	p-value
Gender					
Male	-25.80 (2.91)	-2.09 (3.60)	-23.71 (3.74)	(-31.06, -16.36)	<0.001
Female	-38.06 (2.97)	6.99 (3.90)	-45.05 (4.14)	(-53.19, -36.92)	<0.001
Race					
White	-29.76 (2.48)	2.66 (3.08)	-32.42 (3.09)	(-38.49, -26.36)	<0.001
Non-White	-41.75 (4.47)	-1.79 (5.80)	-39.96 (6.87)	(-53.46, -26.46)	<0.001
Age (years)					
≤19	-28.95 (10.86)	2.88 (11.72)	-31.84 (15.54)	(-62.39, -1.28)	0.041
20 - 29	-33.09 (6.82)	37.78 (11.66)	-70.87 (13.15)	(-96.73, -45.01)	<0.001
30 - 39	-15.92 (6.16)	-12.86 (11.66)	-3.05 (12.90)	(-28.42, 22.31)	0.813
40 - 49	-28.04 (4.46)	3.44 (5.07)	-31.49 (6.29)	(-43.87, -19.11)	<0.001
50 - 59	-32.49 (3.41)	-3.34 (4.55)	-29.15 (5.04)	(-39.06, -19.24)	<0.001
60 - 69	-37.22 (3.71)	0.78 (4.94)	-38.00 (5.50)	(-48.82, -27.18)	<0.001
≥70	-37.05 (5.37)	1.74 (7.71)	-38.79 (8.98)	(-56.45, -21.12)	<0.001
Baseline Low-Density Lipoprotein Cholesterol (mg/dL)					
≤100	-7.26 (8.52)	35.78 (8.78)	-43.04 (8.11)	(-58.99, -27.09)	<0.001
>100 - 200	-25.29 (6.69)	6.05 (6.93)	-31.33 (3.48)	(-38.19, -24.48)	<0.001
>200 - 300	-40.99 (7.74)	-10.27 (8.76)	-30.73 (7.73)	(-45.93, -15.53)	<0.001
>300 - 400	-37.46 (7.86)	-10.15 (10.98)	-27.31 (10.22)	(-47.41, -7.22)	0.008
>400	-40.61 (9.04)	-12.46 (10.38)	-28.14 (9.64)	(-47.10, -9.18)	0.004
Region					
North America and Western Europe	-33.64 (2.86)	-0.04 (3.41)	-33.60 (3.14)	(-39.77, -27.42)	<0.001
Other	-26.27 (4.52)	6.56 (5.84)	-32.83 (6.52)	(-45.66, -20.00)	<0.001

CI, confidence interval; LS, least square; PET, primary efficacy time point, SE, standard error of the mean

A mixed model was run separately for each covariate. Fixed effects included the covariate, treatment, treatment x covariate interaction, and a random effect of study. Least square means are presented.

Source: [TE-MM-SUBGROUP-PDAP3.rtf](#)

Details of Results in Elderly Patients

No elderly patients (≥65 years of age) were enrolled in pivotal study ISIS 301012-CS5. This was not unexpected given the relatively short life expectancy of patients with HoFH (Marais, 2004, *Clin Biochem*).

Ten patients in supportive study MIPO3500108 were ≥65 years of age (2 placebo-treated patients and 8 mipomersen-treated patients). Of the 8 mipomersen-treated patients, 1 discontinued due to an AE after 1 week of study treatment, 1 demonstrated a change in LDL-C of -0.7% at PET, and the other 6 had changes in LDL-C ranging from -30.8% to -70.0% at PET.

Twenty-four patients in supportive study ISIS 301012-CS7 were ≥65 years of age

(7 placebo-treated patients and 17 mipomersen-treated patients). Of the 17 mipomersen-treated patients, 4 discontinued study treatment prematurely, 1 demonstrated a change in LDL-C of -5.2% at PET, and the other 15 had changes in LDL-C ranging from -22.7% to -67.5% at PET.

Fifty-one patients in supportive study ISIS 301012-CS12 were ≥ 65 years of age (18 placebo-treated patients and 33 mipomersen-treated patients in the Full Analysis Set). Of the 33 mipomersen-treated patients, 9 discontinued study treatment prematurely, 1 demonstrated a change in LDL-C of 17.6% at PET, 1 demonstrated a change in LDL-C of 8.9% at PET, 1 demonstrated a change in LDL-C of -9.1% at PET, and the other 21 had changes in LDL-C ranging from -19.1% to -86.4% at PET.

Due to the small numbers of elderly patients in these studies, no clear conclusion can be drawn at this time regarding differences in efficacy in elderly patients as compared with the general population.

Details of Results in Paediatric Patients

The inclusion criteria of ISIS 301012-CS5 allowed the enrolment of children 12 years of age and older. Of the 51 randomised patients, 7 were adolescents (12 to <18 years of age), 3 of whom were randomised to mipomersen and 4 to placebo. Although a dose adjustment was allowed for patients below 50 kg (to 160 mg mipomersen once weekly), all of the mipomersen-treated children in ISIS 301012-CS5 were above 50 kg (range, 55 to 61 kg; between 14 and 16 years of age), so all were treated with 200 mg mipomersen once weekly. During ISIS 301012-CS5, mipomersen resulted in changes in LDL-C from -30.8% to -62.0% in the 3 mipomersen-treated adolescent patients. The percent change in LDL-C in the 4 placebo-treated patients ranged from -7.9% to 43.1%.

After Week 28, the 7 adolescent patients from ISIS 301012-CS5 enrolled in OLE study ISIS 301012-CS6. The 3 patients who were receiving mipomersen in ISIS 301012-CS5 continued to receive 200 mg mipomersen once weekly. The percent change in LDL-C as of their last dose of mipomersen ranged from -35.9% to 3.9% in these 3 patients.

The 4 placebo patients from ISIS 301012-CS5 were assigned to receive mipomersen at 200 mg once weekly (3 patients) or 160 mg once weekly (1 patient at 45.8 kg; 13 years of age) in ISIS 301012-CS6. Changes in LDL-C in these patients as of their last dose of mipomersen ranged from -42.1% to 11.2%.

4.2 Other Special/Subgroup Populations

Results in Statin-Intolerant Patients (Phase 2 Study)

A randomised, double-blind, placebo-controlled, single-centre Phase 2 study (ISIS 301012-CS19) was done to assess the safety and efficacy of mipomersen administration in high-risk statin-intolerant patients (patients unable to tolerate any dose of a statin due to any form of side effects, with the exception of clinically significant alanine aminotransferase [ALT] elevations). The study design was similar to that of the Phase 3 studies. Patients were randomised in a 2:1 manner to receive either placebo (n=12) or mipomersen 200 mg SC (n=22; 21 patients were treated) for 26 weeks, followed by a 24-week follow-up period. Discontinuations were similar between treatment groups: 2/12 placebo patients (16.7%) and 5/22 mipomersen patients (22.7%) discontinued, 4 due to AEs and 1 due to ineligibility. Ten placebo-treated patients and 17 mipomersen-treated patients completed the study.

All of the patients included in the study had been previously treated with statins, but none had statins listed as current concomitant medications. The median baseline LDL-C level was 243.7 mg/dL (6.31 mmol/L) in placebo-treated patients and 241.9 mg/dL (6.26 mmol/L) in mipomersen-treated patients.

Clinically and statistically significant reductions in lipid parameters were seen with mipomersen treatment. The mean percent reductions from baseline in LDL-C and apo B during this Phase 2 study were consistent with, but generally greater than, those observed during the 26-week, double blind treatment periods of the 4 Phase 3 studies.

Summary of Efficacy in ISIS 301012-CS19 (Gravimetric Units) – Full Analysis Set (Phase 2 Study)

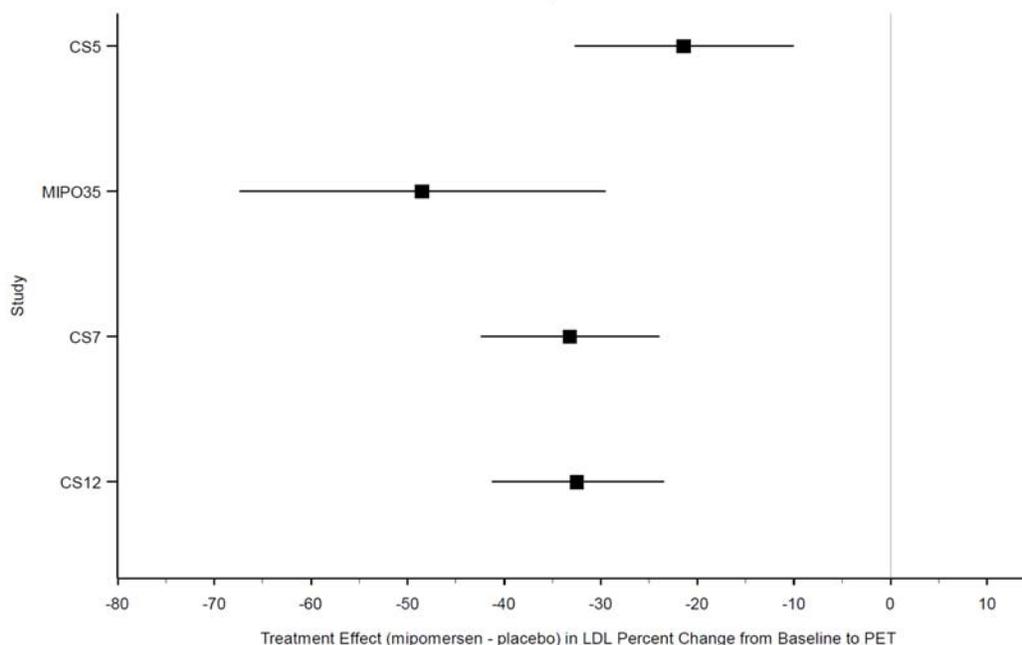
Parameter Time Point	Treatment Arm		p-value
	Placebo (N=12)	Mipomersen (N=21)	
Low-Density Lipoprotein Cholesterol (mg/dL)			
Baseline, mean (SD)	243.7 (65.6)	241.9 (90.8)	<0.001
PET, mean (SD)	236.3 (52.8)	128.3 (74.2)	
% Change from PET, mean (SD)	-2.0 (8.40)	-47.3 (18.43)	
Apolipoprotein B-100 (mg/dL)			
Baseline, median (Q1, Q3)	180 (145, 196)	177 (142, 205)	<0.001
PET, median (Q1, Q3)	173 (143, 187)	96 (66, 110)	
% Change from PET, median (Q1, Q3)	-4.0 (-8.8, 0.5)	-45.8 (-57.5, -34.4)	
Total Cholesterol (mg/dL)			
Baseline, mean (SD)	322.3 (66.2)	318.9 (91.8)	<0.001
PET, mean (SD)	314.5(53.0)	200.1 (77.6)	
% Change from PET, mean (SD)	-1.7 (6.46)	-36.9 (14.66)	
Non-High-Density Lipoprotein Cholesterol (mg/dL)			
Baseline, mean (SD)	273.5 (65.3)	270.1 (92.9)	<0.001
PET, mean (SD)	266.0 (53.0)	147.7 (81.1)	
% Change from PET, mean (SD)	-1.9 (7.06)	-45.6 (18.22)	
Lipoprotein(a) (mg/dL)			
Baseline, mean (SD)	37.3 (76.3)	53.5 (45.8)	<0.001
PET, mean (SD)	41.3 (90.1)	42.3 (48.2)	
% Change from PET, mean (SD)	0.0 (8.65)	-27.1 (31.19)	

PET, primary efficacy time point; Q1, first quartile; Q3, third quartile; SD, standard deviation
 Data presented as mean and SD, with p-values calculated using the 2 sample t-test, unless the result of the Kolmogorov Smirnov test was ≤ 0.05 (indicating non-normal distribution, in which case data are presented as median and interquartile range, with p-values calculated using the Wilcoxon rank- sum test.

Source: [ISIS 301012-CS19 CSR Section 11.1](#)

5. SUMMARY AND CONCLUSIONS

Low-Density Lipoprotein Cholesterol Percent Change from Baseline to Primary Efficacy Time Point Treatment Effects (Difference Between Mipomersen and Placebo Treatment) and 95% Confidence Intervals for Phase 3 Clinical Studies – Full Analysis Set



CS5, Study ISIS 301012-CS5; CS7, Study ISIS 301012-CS7; CS12, Study ISIS 301012-CS12; LDL, low-density lipoprotein cholesterol; MIPO35, Study MIPO3500108; PET, primary efficacy time point

Summary of Efficacy Studies (Gravimetric Units) – Phase 3

Study information	Efficacy Findings			
ISIS301012-CS5				
Phase 3 multicentre, multinational, randomised, double-blind, placebo-controlled, comparative trial of mipomersen 200 mg SC once weekly (N=34) versus placebo (N=17) treatment Patients with HoFH	Endpoint	Placebo (N = 17)	Mipo (N = 34)	p-value
	Mean (SD) Low Density Lipoprotein-Cholesterol (mg/dL)			
	Baseline	400.2 (141.5)	438.9 (138.6)	<0.001
	PET	388.2 (150.5)	326.2 (121.3)	
	% Change from Baseline	-3.3 (17.06)	-24.7 (19.85)	
	Mean (SD) Apolipoprotein B (mg/dL)			
	Baseline	259.2 (84.4)	283.1 (78.4)	<0.001
	PET	252.6 (85.0)	205.4 (70.0)	
	% Change from Baseline	-2.5 (12.56)	-26.8 (17.04)	

26 weeks of treatment 51 randomised 45 completed treatment	Mean (SD) Total Cholesterol (mg/dL)			
	Baseline	460.5 (132.0)	502.4 (144.5)	<0.001
	PET	452.1 (144.6)	389.7 (125.3)	
% Change from Baseline	-2.0 (14.82)	-21.2 (17.69)		
Completed	Mean (SD) Non-High-Density Lipoprotein Cholesterol (mg/dL)			
	Baseline	418.9 (144.5)	464.3 (145.4)	<0.001
	PET	409.1 (156.6)	345.8 (126.6)	
	% Change from Baseline	-2.9 (16.32)	-24.5 (19.17)	
	Mean (SD) Lipoprotein(a) (mg/dL)			
	Baseline	66.3 (53.1)	64.3 (41.0)	<0.001
	PET	61.6 (52.6)	43.8 (32.1)	
	% Change from Baseline	-7.9 (21.87)	-31.1 (23.02)	
	MIPO3500108			
Phase 3 multicentre, multinational, randomised, double-blind, placebo-controlled, comparative trial of mipomersen 200 mg SC once weekly (N=39) versus placebo (N=19) treatment Patients with severe hypercholesterolaemia 26 weeks of treatment	Endpoint	Placebo (N=18)	Mipo (N=39)	p-value
	Mean (SD) Low Density Lipoprotein-Cholesterol (mg/dL)			
	Baseline	249.4 (84.3)	276.1 (72.1)	<0.001
	PET	263.9 (102.0)	174.9 (82.8)	
	% Change from Baseline	12.5 (46.87)	-35.9 (24.71)	
	Mean (SD) Apolipoprotein B (mg/dL)			
	Baseline	182.8 (48.6)	202.1 (49.1)	<0.001
	PET	193.7 (54.2)	126.8 (49.6)	
	% Change from Baseline	11.4 (36.80)	-35.9 (22.95)	
	Mean (SD) Total Cholesterol (mg/dL)			
	Baseline	320.6 (87.2)	356.8 (77.0)	<0.001

Summary of Efficacy Studies (Gravimetric Units) – Phase 3

Study information	Efficacy Findings				
58 randomised 45 completed treatment	PET	341.5 (100.5)	251.5 (82.2)	<0.001	
	% Change from Baseline	11.1 (34.74)	-28.3 (20.43)		
	Mean (SD) Non-High-Density Lipoprotein Cholesterol (mg/dL)				
Completed	Baseline	277.5 (88.3)	305.6 (78.3)	<0.001	
	PET	296.7 (103.8)	198.1 (85.3)		
	% Change from Baseline	14.2 (47.75)	-34.0 (23.80)		
	Mean (SD) Lipoprotein(a) (mg/dL)				
	Baseline	32.4 (28.5)	61.3 (68.4)		
PET	32.1 (28.1)	43.3 (54.3)			

	% Change from Baseline	-1.5 (25.74)	-32.7 (32.98)	<0.001
ISIS301012-CS7				
Phase 3 multicentre, multinational, randomised, double-blind, placebo-controlled, comparative trial of mipomersen 200 mg SC once weekly (N=82) versus placebo (N=41) treatment Patients with HeFH and CAD 26 weeks of treatment 124 randomised 114 completed treatment Completed	Endpoint	Placebo (N=41)	Mipo (N=82)	p-value
	Mean (SD) Low-Density Lipoprotein-Cholesterol (mg/dL)			
	Baseline	142.9 (51.6)	152.9 (48.7)	<0.001
	PET	146.4 (43.4)	103.9 (33.0)	
	% Change from Baseline	5.2 (18.02)	-28.0 (26.99)	
	Mean (SD) Apolipoprotein B (mg/dL)			
	Baseline	126.8 (33.2)	132.8 (33.9)	<0.001
	PET	133.8 (32.6)	95.0 (29.7)	
	% Change from Baseline	7.0 (16.52)	-26.3 (22.16)	
	Mean (SD) Total Cholesterol (mg/dL)			
	Baseline	213.4 (54.6)	225.3 (51.5)	<0.001
	PET	219.0 (49.0)	176.0 (35.9)	
	% Change from Baseline	3.9 (12.84)	-19.4 (19.25)	
	Mean (SD) Non-High-Density Lipoprotein Cholesterol (mg/dL)			
	Baseline	165.3 (54.5)	175.5 (51.1)	<0.001
	PET	168.2 (47.5)	125.2 (37.8)	
	% Change from Baseline	3.7 (16.04)	-25.1 (25.71)	
	Median (Q1, Q3) Lipoprotein(a) (mg/dL)			
Baseline	53 (17, 108)	45 (13, 93)	<0.001	
PET	51 (18, 108)	35 (9, 56)		
% Change from Baseline	0.0 (-8.0, 13.0)	-21.1 (-37.9, 0.0)		

Summary of Efficacy Studies (Gravimetric Units) – Phase 3

Study information	Efficacy Findings			
ISIS301012-CS12				
Phase 3 multicentre, randomised, double-blind, placebo-controlled, comparative trial of mipomersen 200 mg SC once weekly (N=105) versus placebo (N=53) treatment Patients with	Endpoint	Placebo (N=50)	Mipo (N=101)	p-value
	Mean (SD) Low-Density Lipoprotein-Cholesterol (mg/dL)			
	Baseline	122.7 (38.6)	122.6 (31.7)	<0.001
	PET	113.3 (35.1)	75.3 (32.4)	
	% Change from Baseline	-4.5 (24.22)	-36.9 (26.85)	
	Median (Q1, Q3) Apolipoprotein B (mg/dL)			
	Baseline	106 (98, 132)	114 (102, 129)	<0.001
	PET	108 (91, 122)	64 (52, 95)	

hypercholesterolaemia at high risk for cardiovascular events	% Change from Baseline	-1.7 (-12.6, 7.5)	-40.6 (-53.9, -22.6)	<0.001	
	Mean (SD) Total Cholesterol (mg/dL)				
26 weeks of treatment	Baseline	200.0 (42.1)	202.6 (36.8)	<0.001	
	PET	192.2 (38.3)	147.4 (39.9)		
158 randomised	% Change from Baseline	-2.7 (14.58)	-26.4 (18.65)	<0.001	
	Median (Q1, Q3) Non-High-Density Lipoprotein Cholesterol (mg/dL)				
104 completed treatment	Baseline	144 (125, 175)	144 (132, 171)	<0.001	
	PET	140 (115, 165)	90 (67, 116)		
Completed	% Change from Baseline	-1.2 (-13.6, 11.5)	-38.7 (-54.0, -24.2)	<0.001	
	Mean (SD) Lipoprotein(a) (mg/dL)				
	Baseline	51.1 (48.6)	54.3 (57.0)	<0.001	
	PET	49.5 (47.3)	39.6 (47.0)		
	% Change from Baseline	2.3 (28.09)	-24.0 (24.47)	<0.001	
ISIS301012-CS6 (HoFH Patients Only) (Data through 25 March 2011)					
Phase 3 open-label extension for patients who enrolled in ISIS 301012-CS5 or ISIS 301012-CS7	Endpoint Time Point	Value		Percent Change from Baseline	
		n	Mean (SD) or Median (Q1, Q3)	n	Mean (SD) or Median (Q1, Q3)
Mean (SD) Low-Density Lipoprotein-Cholesterol (mg/dL)					
Mipomersen 200 mg SC once weekly	Baseline	38	420.1 (145.8)	--	--
	Week 26	32	336.4 (109.6)	32	-25.07 (-30.7, -19.4)
Patients with HoFH, FH, or HeFH and CAD	Week 52	27	341.0 (126.8)	27	-24.71 (-32.3, -17.2)
	Week 76	10	322.2 (163.0)	10	-32.91 (-51.1, -14.7)
	Week 104	3	254.7 (109.5)	3	-38.47 (-114.1, 37.1)
	Week 130	4	404.4 (174.6)	4	-17.52 (-34.8, -0.2)
Mean (SD) Apolipoprotein B (mg/dL)					

Summary of Efficacy Studies (Gravimetric Units) – Phase 3

Study information	Efficacy Findings				
Up to 104 weeks of treatment 39 HoFH patients enrolled 12 HoFH patients completed treatment as of data cutoff	Baseline	38	273.2 (84.2)	--	--
	Week 26	32	212.2 (64.6)	32	-26.63 (-31.9, -21.4)
	Week 52	27	217.6 (79.2)	27	-25.12 (-33.1, -17.1)
	Week 76	10	199.7 (88.4)	10	-34.41 (-49.2, -19.6)
	Week 104	3	152.0 (62.6)	3	-42.31 (-106.8, 22.2)
	Week 130	4	230.3 (97.8)	4	-29.50 (-44.4, -14.6)
	Mean (SD) Total Cholesterol (mg/dL)				
Baseline	38	483.9 (149.0)	--	--	
Week 26	32	397.4 (111.5)	32	-21.98 (-27.0, -17.0)	

Ongoing	Week 52	27	408.2 (131.2)	27	-20.31 (-27.1, -13.5)	
	Week 76	10	399.9 (163.5)	10	-26.57 (-41.8, -11.3)	
	Week 104	3	315.0 (93.3)	3	-32.63 (-103.2, 37.9)	
	Week 130	4	475.3 (187.8)	4	-14.89 (-26.9, -2.9)	
	Mean (SD) Non-High-Density Lipoprotein Cholesterol (mg/dL)					
	Baseline	38	444.3 (154.8)	--	--	
	Week 26	32	357.8 (115.4)	32	-24.67 (-30.2, -19.2)	
	Week 52	27	368.7 (136.4)	27	-23.18 (-30.7, -15.7)	
	Week 76	10	355.2 (17.0)	10	-30.40 (-47.6, -13.2)	
	Week 104	3	268.3 (114.0)	3	-38.53 (-116.3, 39.3)	
	Week 130	4	437.9 (200.2)	4	-17.99 (-34.6, -1.4)	
	Median (Q1, Q3) Lipoprotein(a) (mg/dL)					
	Baseline	38	50 (26, 65)	--	--	
	Week 26	32	34 (19, 49)	32	-21.6 (-42.2, -4.8)	
	Week 52	27	34 (16, 50)	27	-25.0 (-39.8, -4.9)	
	Week 76	10	31 (26, 49)	10	-26.9 (-38.1, -6.9)	
	Week 104	3	29 (24, 53)	3	17.1 (-55.6, 3.9)	
Week 130	4	35 (30, 38)	4	-25.0 (-32.2, -17.4)		
ISIS301012-CS6 (All Patients) (Data through 30 November 2011)						
Phase 3 open-label extension for patients who enrolled in ISIS 301012-CS5 or ISIS 301012-CS7	Endpoint Time Point	Value		Percent Change from Baseline		
		n	Mean (SD) or Median (Q1, Q3)	n	Mean (SD) or Median (Q1, Q3)	
Mipomersen 200 mg SC once weekly Patients with HoFH, FH, or HeFH and CAD	Mean (SD) Low-Density Lipoprotein-Cholesterol (mg/dL)					
	Baseline	141	232.7 (147.4)	--	--	
	Week 26	130	164.9 (117.9)	130	-28.48 (-31.9, -25.1)	
	Week 52	111	168.3 (121.9)	111	-27.03 (-31.2, -22.8)	
	Week 76	66	144.3 (105.8)	66	-27.32 (-33.0, -21.6)	
	Week 104	53	115.4 (54.2)	53	-28.35 (-34.7, -22.0)	
	Week 130	31	146.6 (94.5)	31	-18.76 (-29.6, -7.9)	

Summary of Efficacy Studies (Gravimetric Units) – Phase 3

Study information	Efficacy Findings				
Up to 104 weeks of treatment	Week 156	5	128.5 (27.1)	5	-19.95 (-49.6, 9.7)
	Week 164	2	102.0 (26.9)	2	-38.40 (-181.9, -105.1)
142 enrolled 17 completed treatment as of data cutoff	Mean (SD) Apolipoprotein B (mg/dL)				
	Baseline	141	175.4 (81.0)	--	--
	Week 26	130	123.7 (65.4)	130	-28.93 (-32.0, -25.8)
	Week 52	111	125.7 (70.8)	111	-28.10 (-32.0, -24.2)
	Week 76	66	110.0 (57.7)	66	-30.33 (-34.7, -26.0)

Ongoing

Week 104	53	92.7 (31.6)	53	-31.4 (-36.9, -25.9)
Week 130	32	115.5 (62.1)	32	-26.46 (-33.8, -19.1)
Week 156	5	100.7 (21.9)	5	-29.83 (-58.3, -1.4)
Week 164	2	80.5 (19.1)	2	-48.26 (-110.4, 13.8)
Mean (SD) Total Cholesterol (mg/dL)				
Baseline	141	302.6 (146.7)	--	--
Week 26	130	231.4 (115.8)	130	-21.67 (-24.4, -18.9)
Week 52	111	236.3 (123.0)	111	-20.36 (-23.9, -16.8)
Week 76	66	214.1 (109.3)	66	-20.05 (-24.6, -15.5)
Week 104	53	185.8 (54.9)	53	-20.14 (-25.4, -14.9)
Week 130	32	233.3 (127.3)	32	-12.67 (-20.9, -4.5)
Week 156	5	197.9 (23.1)	5	-12.25 (-38.3, 13.8)
Week 164	2	165.5 (31.8)	2	-30.17 (-190.6, 130.3)
Mean (SD) Non-High-Density Lipoprotein Cholesterol (mg/dL)				
Baseline	141	256.2 (151.3)	--	--
Week 26	130	184.3 (120.5)	130	-27.22 (-30.4, -24.1)
Week 52	111	189.3 (127.8)	111	-25.37 (-29.5, -21.3)
Week 76	66	166.6 (111.9)	66	-25.01 (-30.4, -19.7)
Week 104	53	134.1 (55.4)	53	-26.70 (-32.7, -20.7)
Week 130	32	180.9 (132.9)	32	-18.07 (-27.6, -8.5)
Week 156	5	147.0 (28.4)	5	-17.33 (-48.7, 14.0)
Week 164	2	114.5 (30.4)	2	-38.67 (-208.8, 131.5)
Median (Q1, Q3) Lipoprotein(a) (mg/dL)				
Baseline	141	46 (14, 93)	--	--
Week 26	130	33 (9, 55)	130	-20.5 (-39.3, -3.6)
Week 52	111	35 (11, 63)	111	-19.0 (-33.3, 0.0)
Week 76	66	40 (15, 56)	66	-17.9 (-33.3, -0.5)
Week 104	53	34 (6, 71)	53	-17.1 (-36.1, 0.0)
Week 130	32	41 (6, 81)	32	-16.7 (-28.2, 0.0)
Week 156	5	71 (63, 100)	5	-22.2 (-33.8, -13.9)
Week 164	2	100 (3, 197)	2	-24.4 (-40.0, -8.8)

CAD, coronary artery disease; HeFH, heterozygous familial hypercholesterolaemia; HoFH, homozygous familial hypercholesterolaemia; PET, primary efficacy time point; Q1, 1st quartile; Q3, 3rd quartile;

SC, subcutaneous; SD, standard deviation; SH, severe hypercholesterolaemia

Source: ISIS 301012-CS5 CSR; MIPO3500108 CSR; ISIS 3010120-CS7 CSR; ISIS 301012-CS12

CSR; ISIS 301012-CS6 CSR Addendum, ISIS 301012-CS6 subset HoFH CSR

Sensitivity analyses of the primary efficacy parameter consisted of the following:

1. Percent change at the lipid assessment closest to 14 days after the last protocol-prescribed

dosing day (i.e., in a 26-week treatment study, this corresponded to the Week 28 assessment). For patients completing 26 weeks of study treatment, these data were identical to that in the PET analysis. However, for patients who discontinued study treatment early, these data could have been substantially after their last dose of study medication. These data were analysed in the same way as PET data (see above) with the exception that no tabulations by site or by category of change were provided.

2. Linear regression analyses and corresponding subgroup tabulations for the following factors: baseline LDL-C, age, sex, and race (e.g., White vs. non-White if supported by adequate distribution of patients). The linear regression analyses consisted of 2 models for each factor; the first model included terms for treatment, factor, and treatment-by-factor interaction while the second model only had terms for treatment and factor. These analyses were not executed for Phase 2 studies because the studies had sample sizes of approximately 8 patients per treatment group so such subgroup analyses could not be reliably interpreted.

3. Robustness of overall findings was assessed by a qualitative comparison to LDL-C percent change from Day 1 to PET (i.e., only a single assessment was used in the baseline determination).

5.1 Statistical Issues and Collective Evidence

Based on my own parametric and nonparametric analyses and the sponsor's results, efficacy results are highly significant showing the efficacy of the drug with respect to LDL-C.

There were no imbalances with respect to baseline factors/covariates. In the pooled analysis, gender by treatment interaction was statistically significant ($p < 0.001$). The treatment effect in females was larger than that seen in males.

5.2 Conclusions and Recommendations

Based on my own parametric and nonparametric analyses and the sponsor's results, efficacy results are highly significant showing the efficacy of the drug with respect to LDL-C.

There were no imbalances with respect to baseline factors/covariates. In the pooled analysis, gender by treatment interaction was statistically significant ($p < 0.001$). The treatment effect in

females was larger than that seen in males.

Japobrata Choudhury, Ph.D.
Mathematical Statistician

Concur: Dr. Sahlroot

APPENDICES

Appendix I

Appendix Table 1

Table 1a
Study ISIS 301012-CS5
Subgroup Analyses of Percent Change from Baseline in LDL-C
Full Analysis Set

	Treatment x covariate interaction P- value	Mean % Change (SE) at PET, Active	Mean % Change (SE) at PET, Placebo	Mean Difference (SE) in % Change, Active - Placebo	95% CI for Mean Difference in % Change, Active - Placebo	Treatment Difference P- value
Gender	0.664					
Male		-24.83 (4.99)	-0.49 (7.31)	-24.34 (8.85)	(-42.14, -6.53)	0.008
Female		-24.53 (4.44)	-5.28 (6.11)	-19.25 (7.55)	(-34.44, -4.05)	0.014
Race	0.380					
White		-24.19 (3.90)	-0.21 (5.29)	-23.98 (6.57)	(-37.20, -10.76)	<0.001
Non-White		-25.78 (6.03)	-13.37 (9.54)	-12.41 (11.29)	(-35.12, 10.30)	0.277
Age (years)	0.056					
<Median		-29.61 (4.38)	3.36 (7.02)	-32.97 (8.27)	(-49.60, -16.33)	<0.001
≥Median		-19.09 (4.64)	-7.98 (5.87)	-11.12 (7.48)	(-26.17, 3.94)	0.144
Baseline LDL-C (mg/dL)	0.599					
<Median		-19.17 (4.88)	-1.75 (5.98)	-17.42 (7.72)	(-32.95, -1.90)	0.029
≥Median		-28.99 (4.34)	-5.54 (7.14)	-23.45 (8.36)	(-40.27, -6.64)	0.007

Note: A mixed model was run separately for each covariate. Fixed effects included the covariate, treatment, and treatment*covariate. Least square means are presented. Apo, B, apolipoprotein B; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein(s); PET, primary efficacy time point; Q1, first quartile; Q3, third quartile; SD, standard deviation. TC, total cholesterol

Data presented as means, with p-values calculated using the 2 sample t-test, unless the result of the Kolmogorov Smirnov test was ≤ 0.05 (indicating non-normal distribution, in which case data are presented as medians, with p-values calculated using the Wilcoxon rank-sum test.

*The percent changes from baseline in the mipomersen group was statistically significant ($p < 0.001$) for all 4 studies.

Table 1b

Study ISIS 301012-CS7
Subgroup Analyses of Percent Change from Baseline in LDL-C
Full Analysis Set

	Treatment x covariate interaction P-value	Mean % Change (SE) at PET, Active	Mean % Change (SE) at PET, Placebo	Mean Difference (SE) in % Change, Active - Placebo	95% CI for Mean Difference in % Change, Active - Placebo	Treatment Difference P-value
Gender	0.051					
Male		-19.97 (3.27)	5.87 (4.37)	-25.84 (5.46)	(-36.65, -15.03)	<0.001
Female		-40.60 (4.09)	3.67 (6.41)	-44.27 (7.61)	(-59.33, -29.21)	<0.001
Race	0.066					
White		-26.78 (2.65)	5.90 (3.84)	-32.68 (4.66)	(-41.91, -23.45)	<0.001
Non-White		-77.72 (16.73)	-4.08 (13.66)	-73.64 (21.60)	(-116.42, -30.86)	<0.001
Age (years)	0.666					
<Median		-26.81 (3.79)	4.35 (5.49)	-31.17 (6.68)	(-44.38, -17.95)	<0.001
≥Median		-29.29 (3.88)	5.95 (5.36)	-35.24 (6.62)	(-48.35, -22.13)	<0.001
Baseline LDL-C (mg/dL)	0.622					
<Median		-18.52 (3.82)	10.51 (4.74)	-29.03 (6.09)	(-41.09, -16.97)	<0.001
≥Median		-35.83 (3.46)	-2.36 (5.64)	-33.47 (6.62)	(-46.57, -20.37)	<0.001

Table 1c

Study ISIS 301012-CS12
Subgroup Analyses of Percent Change from Baseline in LDL-C
Full Analysis Set

	Treatment x covariate interaction P-value	Mean % Change (SE) at PET, Active	Mean % Change (SE) at PET, Placebo	Mean Difference (SE) in % Change, Active - Placebo	95% CI for Mean Difference in % Change, Active - Placebo	Treatment Difference P-value
Gender	0.045					
Male		-32.73 (3.61)	-8.61 (4.79)	-24.11 (6.00)	(-35.97, -12.26)	<0.001
Female		-41.21 (3.65)	1.10 (5.63)	-42.31 (6.71)	(-55.57, -29.05)	<0.001
Race	0.074					
White		-33.47 (2.88)	-5.27 (4.15)	-28.21 (5.06)	(-38.20, -18.22)	<0.001
Non-White		-49.32 (5.46)	-2.21 (7.39)	-47.11 (9.19)	(-65.27, -28.94)	<0.001
Age (years)	0.054					
<Median		-29.82 (4.01)	-7.36 (5.24)	-22.46 (6.60)	(-35.51, -9.42)	<0.001
≥Median		-41.78 (3.32)	-1.93 (5.04)	-39.85 (6.03)	(-51.77, -27.94)	<0.001
Baseline LDL-C (mg/dL)	0.526					
<Median		-31.34 (3.54)	3.55 (4.96)	-34.89 (6.10)	(-46.94, -22.85)	<0.001
≥Median		-42.63 (3.58)	-13.29 (5.16)	-29.33 (6.28)	(-41.74, -16.92)	<0.001

Table 1d

Study MIPO3500108

Subgroup Analyses of Percent Change from Baseline in LDL-C Full Analysis Set

	Treatment x covariate interaction P- value	Mean % Change (SE) at PET, Active	Mean % Change (SE) at PET, Placebo	Mean Difference (SE) in % Change, Active - Placebo	95% CI for Mean Difference in % Change, Active - Placebo	Treatment Difference P- value
Gender	0.001					
Male		-26.97 (7.20)	-14.66 (11.54)	-12.31 (13.60)	(-39.59, 14.96)	0.369
Female		-43.60 (6.66)	29.85 (9.20)	-73.45 (11.36)	(-96.24, -50.66)	<0.001
Race	0.889					
White		-35.53 (5.88)	13.58 (8.72)	-49.11 (10.51)	(-70.20, -28.02)	<0.001
Non-White		-38.10 (13.78)	7.34 (19.49)	-45.45 (23.87)	(-93.33, 2.44)	0.062
Age (years)	0.673					
<Median		-25.59 (7.03)	26.96 (10.48)	-52.55 (12.62)	(-77.87, -27.23)	<0.001
≥Median		-46.80 (7.21)	-1.87 (10.48)	-44.93 (12.73)	(-70.46, -19.41)	<0.001
Baseline LDL-C (mg/dL)	0.270					
<Median		-33.88 (7.77)	23.59 (10.43)	-57.48 (13.01)	(-83.57, -31.39)	<0.001
≥Median		-37.67 (7.20)	-1.27 (11.66)	-36.40 (13.70)	(-63.89, -8.91)	0.010

Appendix II

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

2 ₀ -MOE	2 ₀ -O-(methoxy)-ethyl
ACE	Angiotensin-converting enzyme
AE	Adverse event
ALT	Alanine aminotransferase
Apo	Apolipoprotein
aPTT	Activated partial prothrombin time
ASO	Antisense oligonucleotide
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
AUC _{trough}	Area under the plasma trough concentration-time curve
BMI	Body mass index
BUN	Blood urea nitrogen
CHD	Coronary heart
disease CPK	Creatine

phosphokinase CT	Computed
tomography CVD	Cardiovascular
disease DNA	Deoxyribonucleic
acid	
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
eCRF	Electronic case report form
ELISA	Enzyme-linked immunosorbent assay
FH	Familial hypercholesterolemia
HbA1c	Glycated hemoglobin A 1c
HDL-C	High-density lipoprotein cholesterol
HeFH	Heterozygous familial hypercholesterolemia
HF	High-fat
HMG-CoA	3-hydroxy-3-methylglutaryl co-enzyme A
HoFH	Homozygous familial
hypercholesterolemia	High-sensitivity C-reactive
hsCRP	protein
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
Ig	Immunoglobulin
INR	International normalized ratio
IRB	Institutional Review Board
IVRS	Interactive Voice Response System
LDL	Low-density lipoprotein
LDL-C	Low-density lipoprotein
cholesterol	Low-density lipoprotein receptor
LDL-R	
Lp(a)	Lipoprotein a
MedDRA	Medical Dictionary for Regulatory Activities
MOE	Methoxy-ethyl
MRI	Magnetic resonance
imaging mRNA	Messenger ribonucleic
acid	

non-HDL-C	Non-high-density lipoprotein cholesterol
PET	Primary efficacy time point
PK	Pharmacokinetics
c PT	Prothrombin
time RBC	Red blood cell
SAE	Serious adverse event
SAP	Statistical Analysis
Plan SC	Subcutaneous
SI	International System (of
units) TG	Triglycerides
ULN	Upper limit of normal
VLDL	Very-low-density lipoprotein
VLDL-C	Very-low-density lipoprotein cholesterol
WBC	White blood cell
WHO	World Health Organization

SIGNATURES/DISTRIBUTION LIST (Optional)

CC:

Archival NDA **203568/000**

HFD-510/Dr. Parks

HFD-510/Dr. Colman

HFD-510/ Dr. Craig

HFD-700/Ms. Patrician

HFD-715/Dr. Permutt

HFD-715/Dr. Sahlroot

HFD-715/Dr. Choudhury

J.Choudhury:6-1184: 11/12/12

This review consists of 67 pages.

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/s/

JAPOBRATA CHOUDHURY
11/12/2012

JON T SAHLROOT
11/13/2012



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

Memorandum

Date: 6/20/2012
To: Eileen Craig, MD, Medical Officer
From: Eugenio Andraca-Carrera, Ph.D.
Statistical reviewer
Division of Biometrics 7
Office of Biostatistics

Through: Mat Soukup, Ph.D.
Statistical team leader
Division of Biometrics 7
Office of Biostatistics

Drug: Mipomersen
Sponsor: ISIS Pharmaceuticals
NDA: 203568
Subject: Analysis of broad and narrow SMQs for cardiovascular safety

1. Introduction

Mipomersen is a cholesterol-reducing drug administered by subcutaneous injection. Upon request from the Division of Metabolism and Endocrinology Products (DMEP), we conducted a search of cardiovascular (CV) adverse events included in pre-specified Broad and Narrow MedDRA SMQs in four Phase 3 clinical trials for mipomersen. The Relative Risk was estimated comparing mipomersen to placebo based on the results of these Broad and Narrow CV searches.

This memorandum briefly describes the trials used in analyses, the SMQs included in the Broad and Narrow CV searches, and the estimated Relative Risk for CV events comparing mipomersen to placebo.

2. Clinical Trials

Four Phase 3 clinical trials, identified by DMEP, were used in this analysis: trials **CS5**, **CS7**, **CS12** and **MIPO3500108**. These four trials were randomized, double-blind, placebo controlled and were conducted between September 2007 and October 2010. All subjects randomized to mipomersen received a weekly injection of mipomersen 200 mg. The four trials had a 26 weeks treatment period and a 24-week post treatment follow-up period. The analysis of interest in this document includes only the 26 week treatment

period per the request of the medical officer. Table 1 shows the sample size in the four trials.

Table 1. Sample size by trial

Trial	Sample Size	
	Mipomersen 200mg	Placebo
CS5	34	17
CS7	83	41
CS12	105	53
MIPO108	39	19
Total:	261	130

All information used in this analysis, including randomized treatment, length of treatment period, type of adverse events and date of adverse events were extracted from analysis datasets named ADAE.xpt submitted for each of the four trials of interest.

3. Broad and Narrow SMQ search.

Adverse events were extracted from the variable AEDECOD, labelled “Dictionary-Derived Term”, in files ADAE.xpt. Values of the variable AEDECOD correspond to MedDRA Preferred Terms. Adverse events were classified according to a Broad and a Narrow search of MedDRA SMQs corresponding to cardiovascular adverse events.

Adverse events with Preferred Terms listed in the following MedDRA v14.1 SMQs were included in the “Broad” CV search:

- Haemorrhagic cerebrovascular conditions SMQ
- Ischaemic cerebrovascular conditions SMQ
- Ischaemic heart disease SMQ

Adverse events with Preferred Terms listed in the following MedDRA v14.1 SMQs were included in the “Narrow” CV search:

- Ischaemic cerebrovascular conditions SMQ
- Myocardial infarction SMQ

Note that the SMQs in the “Narrow” search are contained in the SMQs in the “Broad” search. All adverse events in the Broad and Narrow searches were also classified as “Serious” or “Non-Serious”.

4. Statistical Methodology

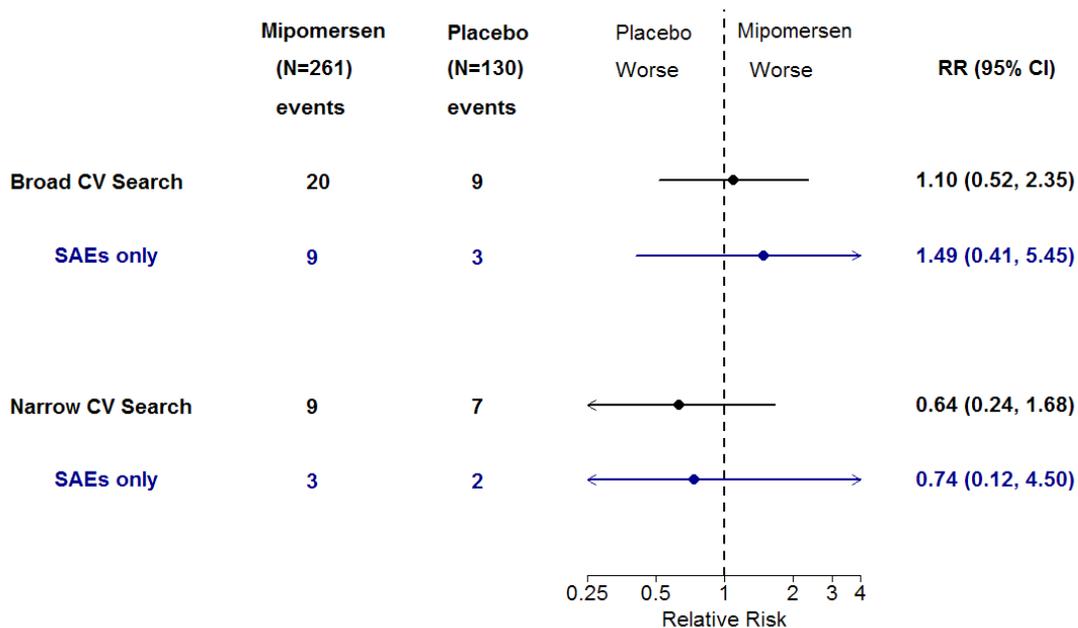
The Mantel-Haenszel Relative Risk of adverse events in the Broad and Narrow CV searches, and its corresponding 95% confidence interval, were estimated comparing mipomersen 200 mg to placebo. A forest plot was produced to summarize the results.

5. Results

There were a total of 20 subjects on mipomersen (N=261) and 9 subjects on placebo (N=130) with a reported adverse event in the “Broad” SMQ search category. There were 9 subjects on mipomersen and 7 subjects on placebo with a reported adverse event in the “Narrow” SMQ search category. Figure 1 shows the estimated Relative Risk and corresponding 95% confidence intervals comparing mipomersen to placebo.

The estimated Relative Risk and 95% CI for the “Broad” CV search were 1.10 (0.52, 2.35). The estimated Relative Risk and 95% CI for the “Narrow” CV search were 0.64 (0.24, 1.68). There was no statistically significant evidence of a difference in risk between mipomersen and placebo in both the Broad and Narrow CV searches. The upper bound of the 95% confidence interval suggests that it may be reasonable to rule out a RR of “Broad” CV events larger than 2.35, and a RR of “Narrow” CV events larger than 1.68. However, note that the estimates of the RR and corresponding 95% confidence intervals reported here are sensitive to small changes in the number of events in either randomized arm, and that the adverse events used in this analysis were not pre-specified and adjudicated. Therefore, these results should be interpreted with caution. If a more precise estimate of the cardiovascular risk of mipomersen is required, a larger study with a pre-specified and adjudicated cardiovascular outcome should be considered. Also, note that the four clinical trials used in this analysis included data up to 26 weeks. These data provide no information on the long-term cardiovascular safety of mipomersen.

Figure 1. Relative Risk of Broad and Narrow Cardiovascular SMQs



Appendix. List of adverse events in the Broad and Narrow CV searches.

Trial	Subject ID	Treatment*	Study Day		Preferred term	MedDRA Code	Serious AE	Broad	Narrow
			End of treatment	Start of AE					
301012-CS05	1500-8881	ISIS 301012 200 mg	190	156	Angina pectoris	10002383	N	1	0
301012-CS05	1523-8309	ISIS 301012 200 mg	192	44	Acute coronary syndrome	10051592	Y	1	1
301012-CS05	1530-8081	ISIS 301012 200 mg	190	141	Blood creatine phosphokinase increased	10005470	N	1	1
301012-CS07	1503-7426	ISIS 301012 200 mg	191	181	Angina pectoris	10002383	Y	1	0
301012-CS07	1505-7023	ISIS 301012 200 mg	128	16	Angina pectoris	10002383	N	1	0
301012-CS07	1506-7324	ISIS 301012 200 mg	190	56	Angina pectoris	10002383	N	1	0
301012-CS07	1578-7165	Placebo	189	189	Coronary artery disease	10011078	Y	1	0
301012-CS07	1578-7437	Placebo	191	170	Carotid artery stenosis	10007687	N	1	1
301012-CS07	1579-7079	ISIS 301012 200 mg	190	98	Myocardial ischaemia	10028600	N	1	0
301012-CS07	1587-7289	ISIS 301012 200 mg	190	190	Electrocardiogram T wave inversion	10014395	N	1	0
301012-CS07	1589-7479	ISIS 301012 200 mg	197	178	Acute myocardial infarction	10000891	Y	1	1
301012-CS07	1589-7479	ISIS 301012 200 mg	197	178	Coronary artery disease	10011078	N	1	0
301012-CS07	1589-7479	ISIS 301012 200 mg	197	197	Infarction	10061216	N	1	1
301012-CS07	1597-7270	Placebo	191	58	Blood creatine phosphokinase increased	10005470	N	1	1
301012-CS07	1623-7247	Placebo	191	15	Blood creatine phosphokinase increased	10005470	N	1	1
301012-CS07	1623-7247	Placebo	191	2	Myocardial ischaemia	10028600	N	1	0
301012-CS12	1535-2369	Placebo	187	110	Acute coronary syndrome	10051592	Y	1	1
301012-CS12	1535-2369	Placebo	187	Unknown ¹	Angina pectoris	10002383	N	1	0
301012-CS12	1547-1420	Placebo	120	112	Acute myocardial infarction	10000891	Y	1	1
301012-CS12	1597-1033	ISIS 301012 200 mg	193	265**	Dysarthria	10013887	N	0	0
301012-CS12	1597-1277	ISIS 301012 200 mg	190	190	Angina pectoris	10002383	N	1	0
301012-CS12	1633-2169	ISIS 301012 200 mg	190	113	Blood creatine phosphokinase increased	10005470	N	1	1
301012-CS12	1636-1254	ISIS 301012 200 mg	190	334**	Blood creatine phosphokinase increased	10005470	N	1	1
301012-CS12	1646-1374	ISIS 301012 200 mg	102	326**	Blood creatine phosphokinase increased	10005470	N	1	1
301012-CS12	1660-1242	ISIS 301012 200 mg	190	134	Carotid artery stenosis	10007687	N	1	1
301012-CS12	1664-2055	Placebo	190	273**	Acute myocardial infarction	10000891	Y	1	1
301012-CS12	1681-1008	ISIS 301012 200 mg	188	14	Blood creatine phosphokinase increased	10005470	N	1	1
301012-CS12	1681-1008	ISIS 301012 200 mg	188	216**	Blood creatine phosphokinase increased	10005470	N	1	1
301012-CS12	1681-1095	ISIS 301012 200 mg	114	114	Angina pectoris	10002383	Y	1	0
301012-CS12	1681-1358	ISIS 301012 200 mg	142	67	Angina unstable	10002388	Y	1	0
301012-CS12	1681-2132	ISIS 301012 200 mg	191	325**	Acute myocardial infarction	10000891	Y	1	1
301012-CS12	1681-2132	ISIS 301012 200 mg	191	127	Angina pectoris	10002383	N	1	0
301012-CS12	1681-2132	ISIS 301012 200 mg	191	174	Coronary artery disease	10011078	Y	1	0
301012-CS12	1681-2358	ISIS 301012 200 mg	190	338**	Blood creatine phosphokinase increased	10005470	N	1	1

301012-CS12	1682-1256	Placebo	190	1	Blood creatine phosphokinase increased	10005470	N	1	1
301012-CS12	1682-1362	Placebo	190	-7**	Blood creatine phosphokinase increased	10005470	N	1	1
MIPO3500108	1010-1005	Placebo	180	57	Blood creatine phosphokinase increased	10005470	N	1	1
MIPO3500108	3000-1046	ISIS 301012 200 mg	191	159	Angina unstable	10002388	Y	1	0
MIPO3500108	3000-1046	ISIS 301012 200 mg	191	Unknown ²	Cerebrovascular accident	10008190	N	1	1
MIPO3500108	3002-1027	ISIS 301012 200 mg	190	158	Acute myocardial infarction	10000891	Y	1	1
MIPO3500108	3002-1027	ISIS 301012 200 mg	190	205**	Acute myocardial infarction	10000891	Y	1	1
MIPO3500108	3002-1031	Placebo	190	77	Angina pectoris	10002383	N	1	0
MIPO3500108	5002-1056	ISIS 301012 200 mg	199	100	Angina pectoris	10002383	Y	1	0
MIPO3500108	5002-1056	ISIS 301012 200 mg	199	213**	Cerebrovascular accident	10008190	Y	1	1
MIPO3500108	5002-1056	ISIS 301012 200 mg	199	183	Prinzmetal angina	10036759	Y	1	0
MIPO3500108	5002-1056	ISIS 301012 200 mg	199	190	Prinzmetal angina	10036759	Y	1	0
MIPO3500108	6000-1032	ISIS 301012 200 mg	190	3	Angina pectoris	10002383	N	1	0
MIPO3500108	6000-1032	ISIS 301012 200 mg	190	10	Angina pectoris	10002383	N	1	0
MIPO3500108	6000-1032	ISIS 301012 200 mg	190	161	Angina pectoris	10002383	N	1	0
MIPO3500108	6000-1032	ISIS 301012 200 mg	190	17	Blood creatine phosphokinase increased	10005470	N	1	1
MIPO3500108	6000-1032	ISIS 301012 200 mg	190	85	Coronary artery disease	10011078	N	1	0
MIPO3500108	6000-1032	ISIS 301012 200 mg	190	162	Coronary artery disease	10011078	N	1	0

*Mipomersen is referred to as "ISIS 301012 200 mg" in the clinical trials datasets.

**These events occurred outside of the treatment period and are not included in the analysis.

¹This event may be excluded from analyses since subject 1535-2369 had one other reported adverse event that meets the requirements for Serious, Broad and Narrow adverse events.

²The reported date for this event is "2010-04". Since the last treatment date reported for this subject was "2010-04-28"; this event occurred either during the treatment period or within 2 days of the last treatment date. Therefore we considered this event as occurring within the treatment period.

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/s/

EUGENIO ANDRACA-CARRERA
07/02/2012

MATTHEW J SOUKUP
07/02/2012
Concur

STATISTICS FILING CHECKLIST FOR A NEW NDA/BLA

NDA Number: 203568

Applicant: Genzyme Corporation

**Drug Name: Kynamro™
(mipomersen sodium)
Solution for Injection**

NDA/BLA Type: Standard

Submission Date: 03/29/2012

NDA/BLA Serial Number: 000

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	NA	Comments
1	Index is sufficient to locate necessary reports, tables, data, etc.	X			
2	ISS, ISE, and complete study reports are available (including original protocols, subsequent amendments, etc.)	X			
3	Safety and efficacy were investigated for gender, racial, and geriatric subgroups investigated (if applicable).	X			
4	Data sets in EDR are accessible and do they conform to applicable guidances (e.g., existence of define.pdf file for data sets).	X			

IS THE STATISTICAL SECTION OF THE APPLICATION FILEABLE?

 Yes

If the NDA/BLA is not fileable from the statistical perspective, state the reasons and provide comments to be sent to the Applicant.

Studies Submitted

The pivotal Study:

Safety and efficacy	ISIS 301012-CS5 (5.3.5.1)	Safety and efficacy	Phase 3, double-blinded, placebo-controlled	Mipomersen 200 mg (or placebo) SC once weekly (160 mg once weekly for patients weighing <50 kg)	51	Homozygous familial hypercholesterolaemia	26 weeks	Complete; Full CSR
---------------------	---	---------------------	---	---	----	---	----------	--------------------

In addition to the above pivotal study, data from 3 supportive Phase 3 studies in patients with related conditions ([MIPO03500108](#), in patients with Severe HeFH; [ISIS 301012-CS7](#), in patients with HeFH and CAD; and [ISIS 301012-CS12](#), in patients with hypercholesterolaemia at high risk for CHD) have also been presented.

Studies to be Reviewed

File name: 5_Statistics Filing Checklist for a New NDA_BLA110207

STATISTICS FILING CHECKLIST FOR A NEW NDA/BLA

The Pivotal Study [ISIS 301012-CS5](#)

Safety and efficacy	ISIS 301012-CS5 (5.3.5.1)	Safety and efficacy	Phase 3, double-blinded, placebo-controlled	Mipomersen 200 mg (or placebo) SC once weekly (160 mg once weekly for patients weighing <50 kg)	51	Homozygous familial hypercholesterolaemia	26 weeks	Complete; Full CSR
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Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Content Parameter (possible review concerns for 74-day letter)	Yes	No	NA	Comment
Designs utilized are appropriate for the indications requested.	x			
Endpoints and methods of analysis are specified in the protocols/statistical analysis plans.	x			
Interim analyses (if present) were pre-specified in the protocol and appropriate adjustments in significance level made. DSMB meeting minutes and data are available.			x	
Appropriate references for novel statistical methodology (if present) are included.			x	
Safety data organized to permit analyses across clinical trials in the NDA/BLA.				Deferred to Clinical
Investigation of effect of dropouts on statistical analyses as described by applicant appears adequate.				To be requested as needed

Further Information Request in 74-day letter

Provide the following with **discussion and conclusion** for the primary efficacy variable. If they are already in the NDA, please provide the location. The number of patients is an important part of the information.

1. A graph for the percent of patients discontinuing (or continuing) over time by treatment group and on the same page or graph, a similar graph for the percent of patients discontinuing over time due to adverse effects.
2. Graphs for responses over time for the **completer set**, by treatment arm.

File name: 5_Statistics Filing Checklist for a New NDA_BLA110207

STATISTICS FILING CHECKLIST FOR A NEW NDA/BLA

3. Investigation of effect of dropouts on statistical analyses

Sensitivity analyses for the handling of missing data, in addition to the analyses you provided in the NDA.

The sensitivity analyses may be based on parametric models, for example, Mixed Model Repeated Measures or Multiple Imputation.

The effects of dropouts on observed cases (OC) and LOCF results should be investigated graphically. A simple tool is a plot of efficacy results over time for each (separate) cohort (depending on the interval of dropout) for (1) OC and (2) LOCF populations. Please make sure that the graph does not become clumsy (i.e., choose location and scale so that the curves are distinguishable and use different colors). However, you are always welcome to present additional graphs or methods which you think provide more reasonable depictions of the data. The number of patients for each case should be provided.

4. Please provide summary results of a thorough investigation of confounding and interaction effects (but protocol-mentioned primary analysis remains the primary analysis), if you have not already done so. Also, include covariation and interaction p-values. Provide treatment comparison p-values for each level of important subgroups. The number of patients for each case should be provided.

5. For screening and exploratory purposes (not confirmatory), as reviewers, we would like to see (a) 2-sided p-values for all baseline pair-wise comparisons (between treatment groups) on baseline status, demographics, and other prognostic variables.

Japobrata Choudhury, Ph.D.

5-14-12

Reviewing Statistician

Date

Jon T. Sahlroot, Ph.D.

Supervisor/Team Leader

Date

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/s/

JAPOBRATA CHOUDHURY
05/21/2012

JON T SAHLROOT
05/21/2012