

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

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**STATISTICAL REVIEW(S)**



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

## ADDENDUM

**NDA/BLA Serial Number:** NDA 204-410 SN 0000

**Drug Name:** Macitentan

**Indication(s):** Pulmonary Arterial Hypertension

**Applicant:** Acetlion

**Date(s):** 10/19/2012

**Review Priority:** Standard

**Biometrics Division:** Division of Biometrics I

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**Keywords:**  
Mortality

This addendum focuses on the number of mortality events reported by the sponsor. It was noticed that the analysis on time to death at EOT+7 days had 19 deaths in placebo and 14 deaths in macitentan 10 mg group. But there were only 18 adjudicated deaths in placebo and 16 in macitentan 10 mg at any time up to EOT+7 days. Upon further review, the reviewer found two adjudicated death events in macitentan 10 mg group occur beyond EOT + 7 days and one death event that was unadjudicated but occurred within EOT + 7 days. This is consistent with what the sponsor reported in their response to information request submitted on September 24, 2013.

<b>Analysis</b>	<b>Placebo</b>	<b>Macitentan 10 mg</b>	<b>Comment/Explanation/Sources</b>
Primary analysis: Death as the 1 <sup>st</sup> CEC-confirmed event	17	16	Sponsor's primary analysis. Only deaths occurred as the first event were counted.
Total number of all CEC-confirmed deaths	18	16	Patient 13107 in placebo group had two events before EOT + 7 days: one CEC-confirmed worsening of PAH and a CEC-confirmed death occurred one day after EOT.
Secondary endpoint analysis: Total number of deaths at EOT + 7 days used for the secondary endpoint analysis	19	14	Patient 15725 in placebo group was included in the count of deaths for this analysis since this patient experienced a CEC-confirmed worsening event resulting in death one day after EOT. This accounts for the 19 deaths in this analysis.  Patient 13357 and patient 10736 in macitentan 10 mg group died on EOT + 8 days and EOT + 22 days, respectively. Both deaths occurred beyond EOT + 7 days and were excluded from this analysis

[source: sponsor's response to information request submitted on September 24, 2013, verified by the reviewer]

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/s/  
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10/04/2013

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U.S. Department of Health and Human Services  
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# STATISTICAL REVIEW AND EVALUATION

## CLINICAL STUDIES

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**Project Manager:** Dan Brum /Ed Fromm

**Keywords:**

Missing data, early discontinuation, on-treatment analysis, sensitivity analyses

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## 1. EXECUTIVE SUMMARY

The application of macitentan in treating patients with pulmonary arterial hypertension (PAH) included a single phase III study AC-055-302/SERAPHIN. The primary endpoint of the study was a composite morbidity/mortality endpoint with multiple components, including death, lung transplantation, initiation of prostanoids, atrial septostomy and other worsening of PAH. The primary endpoint showed highly significant results. However, it was driven by the single component “other worsening of PAH” with no effect shown for mortality.

“Other worsening of PAH” was defined with three criteria: 15% decrease in 6MWD, worsening of PAH symptoms in terms of WHO FC or right heart failure, and need for new PAH treatment. 54 subjects with CEC-adjudicated “other worsening of PAH” events had some deviation in measuring the second confirmatory 6MWD. Most of these subjects did not take the second 6MWT and the decision of qualifying the events were based on written justifications from investigators. The distribution of these subjects in three treatment arms was relatively even. Excluding these events did not affect much the study results. This provides some assurance for the analysis results.

The subjects were followed for the primary events until the end of treatment (EOT) + 7 days instead of the end of study (EOS). As a result, 93 subjects (33 in placebo group, 26 in macitentan 3 mg group and 34 in macitentan 10 mg group) discontinued treatment early and censored at EOT + 7 days without any primary endpoint events. The sponsor and the reviewer performed a number of sensitivity analyses to assess the impact of the early censoring rule on the study results. At least 18 more events out of the 34 early censored subjects in the macitentan 10 mg group will be needed to change the p-value of logrank test to 0.005. Other sensitivity analyses showed consistent results overall.

To address subject early discontinuation, a time to early discontinuation analysis was performed using early discontinuation as the endpoint event to compare macitentan groups with placebo. The subjects in macitentan groups stayed significantly longer on treatment than placebo group.

On average, about 15% subjects had missing 6MWD measurements at Month 6. The percentage of missing was higher in placebo group (21%) and lower in macitentan groups (13% in low dose group and 12% in high dose group). The subjects in macitentan 10 mg group and macitentan 3 mg group on average had 22 meter and 17 meter improvement in 6-minute walk distance (6MWD) at Month 6, respectively, when compared to placebo. The reviewer also examined the 6MWD at Month 3, which had less missing data (6%). The results in 6MWD at Month 3 were consistent with the results in 6MWD at Month 6.

The subgroup analysis by region showed that the treatment effect of macitentan groups was trending in the wrong direction in US. US had an extreme low primary event rate in the placebo group (17% versus an average of 49% in other regions), while the event rate in the treatment group seemed comparable in US and OUS. The 6MWD at Month 6 also trended in the wrong

direction for US. Caution needs to be taken in interpreting this finding due to very small sample size in US.

Overall, the results in SERAPHIN trial appear to support the efficacy of macitentan 10 mg.

## 2. INTRODUCTION

### 2.1 Overview

Macitentan (ACT-064992) is an orally active, dual endothelin (ET) receptor antagonist. The indication that sponsor is seeking is to use 10 mg macitentan (once daily) for the long-term treatment of pulmonary arterial hypertension (PAH) (b) (4). The clinical evidence for the efficacy and safety of macitentan in the treatment of patients with PAH is derived from a single phase III study AC-055-302/SERAPHIN. This was a pivotal placebo-controlled, multi-national study, which enrolled 742 patients with symptomatic PAH, randomized in a 1:1:1 ratio to macitentan 3 mg o.d., macitentan 10 mg o.d., or placebo. The study included a pre-randomization screening period (up to 28 days) followed by a treatment period from randomization to the end of double-blind study treatment (EOT). This study was designed as an event-driven trial and the sponsor planned to collect 285 primary events. The sponsor declared the end-of-study (EOS) and initiated the EOS procedure on January 30, 2012.

### 2.2 Data Sources

The sponsor's electronic data is stored under the directory <\\cdsesub5\EVSPROD\NDA204410\0000\m5\datasets\ac-055-302>.

Specifically, the derived datasets are under the directory <\\cdsesub5\EVSPROD\NDA204410\0000\m5\datasets\ac-055-302\analysis\legacy\datasets> and raw datasets are stored under the directory <\\cdsesub5\EVSPROD\NDA204410\0000\m5\datasets\ac-055-302\tabulations>

The sponsor's clinical study report can be found in the directory <\\cdsesub5\EVSPROD\NDA204410\0000\m5\53-clin-stud-rep\535-rep-effic-safety-stud\pah\5351-stud-rep-contr\ac-055-302>

## 3. STATISTICAL EVALUATION

### 3.1 Data and Analysis Quality

The reviewer was able to reproduce the results of the primary analysis and secondary analyses. The applicant submitted the tabulation datasets used to derive the primary analysis dataset and the reviewer was able to trace how the primary endpoint was derived.

### 3.2 Evaluation of Efficacy

#### Study Design and Endpoints

This was a multicenter, double-blind, randomized, placebo-controlled, parallel group, event-driven, Phase 3 study. A total of 742 patients were randomized (1:1:1 ratio) to the three treatment groups, i.e., macitentan 3 mg (250 patients), macitentan 10 mg (242 patients), or placebo (250 patients). The study included a screening period (up to 28 days) for the assessment of patients' eligibility, followed by a treatment period from randomization to EOT visit. For patients who prematurely discontinued double-blind treatment, EOT occurred earlier and these patients could be treated with macitentan 10 mg if they met the criteria for enrollment in the open-label extension study or with any available therapy between EOT and EOS.

The primary endpoint was defined as time from start of study treatment to the first morbidity or mortality event up to EOT. The first composite event included:

- Death, or onset of a treatment-emergent AE with a fatal outcome occurring within 4 weeks of study treatment discontinuation, or
- Atrial septostomy or hospitalization for atrial septostomy, or
- Lung transplantation or hospitalization for lung transplantation, or
- Initiation of intravenous (i.v.) or subcutaneous (s.c.) prostanoids (e.g., epoprostenol, treprostinil) or hospitalization for initiation of i.v. or s.c. prostanoids, or
- Other worsening of PAH

Other worsening of PAH was defined by the combined occurrence in a patient of all the following three events:

- At least 15% decrease in the 6-minute walk distance (6MWD) from baseline, confirmed by two 6MWTs, performed on separate days, within 2 weeks of each other.

AND

- Worsening of PAH symptoms that included at least one of the following:
  - Increase in World Health Organization (WHO) functional class (FC), or no change in patients in WHO Class IV at baseline
  - Appearance or worsening of signs/symptoms of right heart failure that did not respond to optimized oral diuretic therapy

AND

- Need for new treatment(s) for PAH that included the following:
  - Oral or inhaled prostanoids (e.g., iloprost)

- Oral phosphodiesterase inhibitors (e.g., sildenafil)
- Endothelin receptor antagonists (ERAs) (e.g., bosentan, ambrisentan) only after discontinuation of the study treatment
- Intravenous diuretics

The secondary endpoints were

- Change in 6MWD from baseline to Month 6
- Proportion of patients with improvement in modified WHO FC from baseline to Month 6
- Time to death due to PAH or hospitalization for PAH up to EOT that included
  - Death due to PAH (as adjudicated by the CEC) up to EOT + 7 days, or
  - Onset of a treatment emergent AE with a fatal outcome due to PAH occurring up to 4 weeks after EOT, or
  - Hospitalization for PAH up to EOT + 7 days
- Time to death of all causes up to EOT + 7 days or occurrence of onset of a treatment emergent AE with a fatal outcome up to EOT + 4 weeks.
- Time to death of all causes up to EOS (this endpoint was changed from an exploratory endpoint to a secondary endpoint)

### Patient Disposition, Demographic and Baseline Characteristics

A total of 955 patients were screened. 742 patients from 151 centers in 39 countries were randomized in a 1:1:1 ratio to the macitentan 3 mg (n = 250), macitentan 10 mg (n = 242) and placebo groups (n = 250). A total of 590 patients (79.5%) completed the study. 16.9% patients in the macitentan 10 mg group, 22.4% patients in the macitentan 3 mg group and 22.0% patients in the placebo group prematurely discontinued the study (**Table 1**).

Table 1 Reasons for Discontinuation of Study

Reason for discontinuation	Placebo N=250		Macitentan 3 mg N=250		Macitentan 10 mg N=242	
	No.	%	No.	%	No.	%
Total patients with at least one reason	55	22.0%	56	22.4%	41	16.9%
Death	44	17.6%	47	18.8%	34	14.0%
Withdrawal of subject's consent	3	1.2%	6	2.4%	4	1.7%
Lost to follow-up	7	2.8%	3	1.2%	2	0.8%
Administrative reason	1	0.4%	-		1	0.4%

[Source: Sponsor's Clinical Study Report Table 60, verified by the reviewer]

The proportion of patients who discontinued study treatment was 44.2% in the macitentan 10 mg group, 47.2% in the macitentan 3 mg group, and 59.4% in the placebo group. A morbidity event followed by enrollment in the open label treatment was the most frequent reason for discontinuation of study treatment in all three groups (**Table 2**).

Table 2 Reasons for Discontinuation of Treatment

Preferred term	Placebo N=249		Macitentan 3 mg N=250		Macitentan 10 mg N=242	
	No.	%	No.	%	No.	%
Total patients with at least one reason	148	59.4%	118	47.2%	107	44.2%
DISEASE PROGRESSION LEADING TO OL	80	32.1%	57	22.8%	50	20.7%
ADVERSE EVENT	31	12.4%	34	13.6%	26	10.7%
DEATH	12	4.8%	10	4.0%	10	4.1%
WITHDRAWAL FROM TREATMENT	11	4.4%	7	2.8%	12	5.0%
ADMINISTRATIVE/OTHER	5	2.0%	2	0.8%	5	2.1%
WITHDRAWAL OF CONSENT	1	0.4%	3	1.2%	4	1.7%
LOST TO FOLLOW-UP	3	1.2%	2	0.8%	-	-
TREATMENT FAILURE	3	1.2%	2	0.8%	-	-
ADMINISTRATION OF FORBIDDEN DRUG	2	0.8%	1	0.4%	-	-

[Source: Sponsor's Clinical Study Report Table 11, verified by the reviewer]

There were over 70% female patients in the trial and the median age for the trial population was approximately 45 years (Table 3). The patients were predominantly Caucasian or Asian.

Table 3 Patient Demographics

	Placebo N=250	Macitentan 3 mg N=250	Macitentan 10 mg N=242	All patients N=742
SEX [n (%)]				
n	249	248	242	739
Males	65 26.1%	61 24.6%	48 19.8%	174 23.5%
Females	184 73.9%	187 75.4%	194 80.2%	565 76.5%
AGE (years)				
n	249	248	242	739
Mean	46.7	44.5	45.5	45.6
Standard deviation	17.03	16.26	14.99	16.13
RACE [n (%)]				
n	249	248	242	739
Caucasian/white	131 52.6%	137 55.2%	135 55.8%	403 54.5%
Black	8 3.2%	5 2.0%	6 2.5%	19 2.6%
Asian	71 28.5%	69 27.8%	65 26.9%	205 27.7%
Hispanic	37 14.9%	37 14.9%	35 14.5%	109 14.7%
Other	2 0.8%	-	1 0.4%	3 0.4%
LOCATION [n (%)]				
n	249	248	242	739
US	23 9.2%	25 10.1%	19 7.9%	67 9.1%
Non-US	226 90.8%	223 89.9%	223 92.1%	672 90.9%
REGION [n (%)]				
n	249	248	242	739
North America	30 12.0%	30 12.1%	23 9.5%	83 11.2%
Western Europe/Israel	50 20.1%	41 16.5%	48 19.8%	139 18.8%
Eastern Europe/Turkey	59 23.7%	63 25.4%	62 25.6%	184 24.9%
Asia	68 27.3%	70 28.2%	68 28.1%	206 27.9%
Latin America	42 16.9%	44 17.7%	41 16.9%	127 17.2%

[Source: Sponsor's Clinical Study Report Table 13, verified by the reviewer]

**Table 4** summarizes the baseline characteristics of the trial population. The mean time from PAH diagnosis to randomization in the study population was 2.7 years. Baseline mean 6MWD

was approximately 360 m. About 52% of patients were in WHO FC II and 46% of patients were in WHO FC III. Very few patients were in WHO FC IV or WHO FC I.

Table 4 Baseline Characteristics

	Placebo N=250	Macitentan 3 mg N=250	Macitentan 10 mg N=242	All patients N=742
Time from PAH diagnosis (days)				
n	247	247	241	735
Mean	942	1079	951	991
Standard deviation	1362.0	1659.1	1325.1	1456.9
6mn Walk Test (m) (absolute)				
n	249	248	242	739
Mean	352.4	364.1	362.6	359.6
Standard deviation	110.62	95.52	93.21	100.15
WHO functional class [n (%)]				
n	249	248	242	739
I	-	-	1 0.4%	1 0.1%
II	129 51.8%	138 55.6%	120 49.6%	387 52.4%
III	116 46.6%	105 42.3%	116 47.9%	337 45.6%
IV	4 1.6%	5 2.0%	5 2.1%	14 1.9%
Concomitant PAH therapy [n (%)]				
n	249	248	242	739
No	95 38.2%	85 34.3%	88 36.4%	268 36.3%
Yes	154 61.8%	163 65.7%	154 63.6%	471 63.7%
Sildenafil	140 56.2%	146 58.9%	140 57.9%	426 57.6%
Tadalafil	2 0.8%	3 1.2%	2 0.8%	7 0.9%
Vardenafil	8 3.2%	5 2.0%	8 3.3%	21 2.8%
Iloprost	3 1.2%	13 5.2%	10 4.1%	26 3.5%
Beraprost	4 1.6%	5 2.0%	6 2.5%	15 2.0%
Treprostinil	-	1 0.4%	-	1 0.1%

[Source: Sponsor's clinical study report Table 14, verified by the reviewer]

471 patients took PAH background therapy at baseline. Among them, 25 patients were taking two PAH background therapy at baseline. **Table 5** shows the percentage of patients taking various PAH background therapy breaking down by region.

Table 5 Various PAH Background Therapy by Region

	North Am	Western EU	Eastern EU	Asia	Latin Am
Beraprost	0	0	0	15 (9.2%)	0
Iloprost	1 (1.5%)	8 (7.8%)	6 (9.0%)	7 (4.3%)	4 (4.2%)
Sildenafil	64 (95.5%)	95 (92.2%)	55 (82.1%)	121 (73.8%)	91 (95.8%)
Tadalafil	1 (1.5%)	0	2 (3.0%)	4 (2.4%)	0
Treprostinil	1 (1.5%)	0	0	0	0
Vardenafil	0	0	4 (6.0%)	17 (10.4%)	0
Total	67 (100%)	103 (100%)	67 (100%)	164 (100%)	95 (100%)

## Statistical Methodologies

To keep the study-wise type-I error to a two-sided 0.01 level, each comparison of active dose versus placebo was tested at a nominal type-I error level of 0.005 (two-sided) using Bonferroni's approach. The secondary endpoints were analyzed hierarchically in the following order.

- Change from baseline to Month 6 in 6MWD
- Change from baseline to Month 6 in modified WHO FC
- Time to death due to PAH or hospitalization for PAH
- Time to death of all causes up to EOT + 7 days
- Time to death of all causes up to EOS

Note that time to all-cause mortality by the end of study was an exploratory endpoint and was changed to the last secondary endpoint prior to unblinding in protocol amendment 3.

The primary and secondary analyses were based on all-randomized set, which included all randomized patients, whether or not they received study drug.

The Wilcoxon rank sum test was used for the analysis of change in 6MWD from baseline to Month 6. The proportion of patients who had an improvement in WHO FC from baseline to Month 6 was analyzed using the Fisher's exact test. Time-to-event analyses were analyzed using logrank test.

A total of 285 events were determined to be needed to detect a hazard ratio of 0.5472 for macitentan versus placebo with a nominal type-I error of 0.005 (two-sided) for each dose group of macitentan (3 mg and 10 mg) and 90% power using the logrank test. In June 2009, the sponsor detected lower than expected overall event rate based on blinded assessment and increased the sample size from 525 patients to 699 patients. The total number of events remained the same. This was reflected in protocol amendment 3 in September 2009. Time to all-cause mortality by the end of study was also changed from an exploratory endpoint to the last secondary endpoint prior to unblinding.

To handle the missing data in the analysis on 6MWD, the last available post-baseline value obtained up to the last day of the Month 6 window (i.e. the earliest day between study day 270 and EOT plus 7 days) was carried forward to impute the missing value unless one of the following applies:

- If the patient died before or on the last day of the Month 6 window, a distance of 0 meter was imputed for the missing values.
- If the patient experienced a confirmed CEC event but did not die up to the last day of the Month 6 window and had no 6MWD value between the occurrence of the event and the last day of the Month 6 window (inclusive), the 25th percentile of the ordered distribution of available 6MWD values in the same analysis set was used for the imputation value. The available values were taken from Month 6 for the patients with 6MWD data in the Month 6 window.

In the case of missing values for the WHO FC, the last available post-baseline value obtained up to the last day of the Month 6 window was carried forward unless the patient experienced a morbidity/mortality event or dies up to the last day of the Month 6 window and has no value between the occurrence of the event and the last day of the Month 6 window. In this case, WHO FC IV is imputed.

## Results and Conclusions

### Primary analysis

In total, the CEC adjudicated 341 primary events in 313 patients and confirmed 296 events in 287 patients. Majority of these morbidity/mortality events were “other worsening of PAH”.

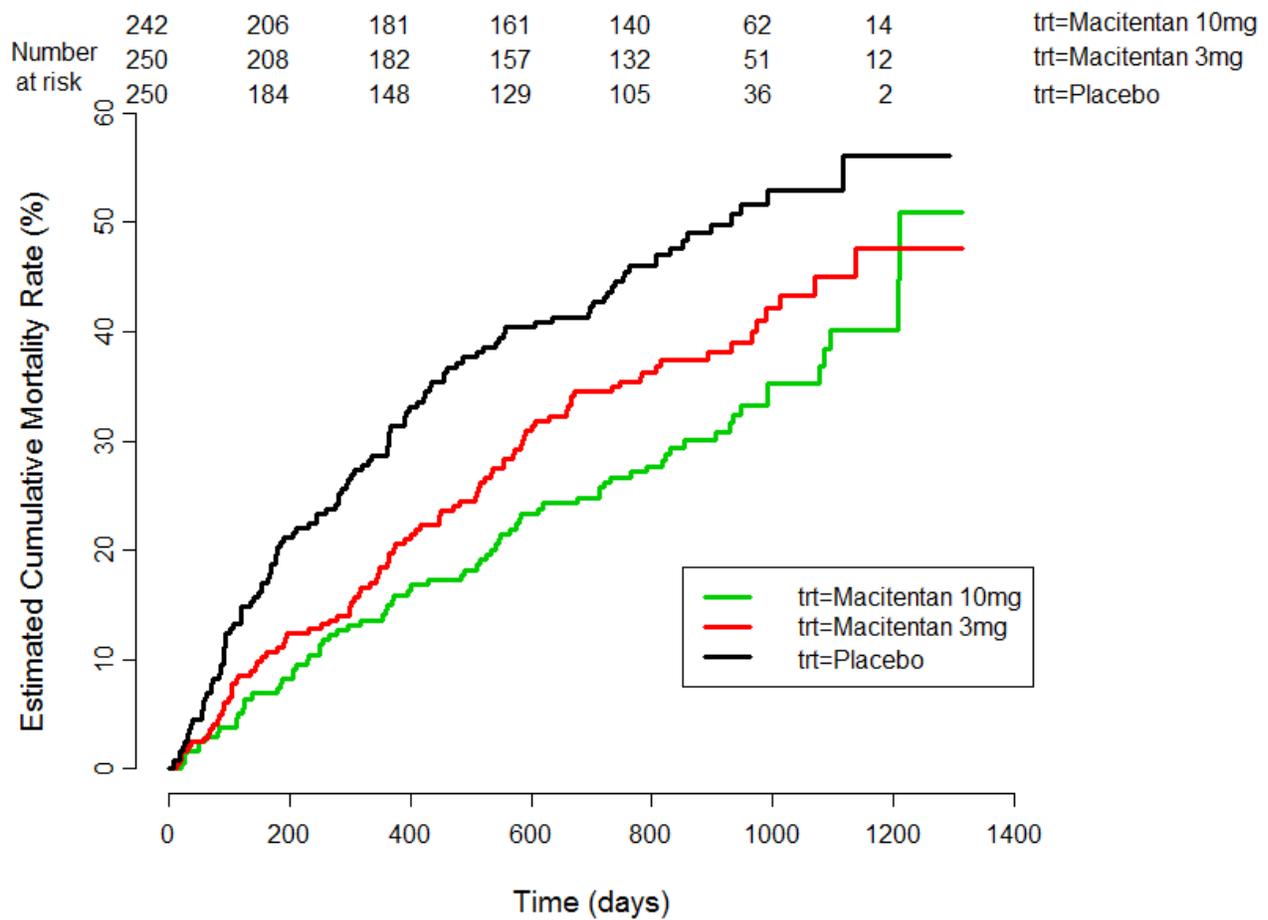
Table 6 Summary on Components of Primary Endpoint Events

	Placebo		Macitentan 3 mg		Macitentan 10 mg	
	N=250		N=250		N=242	
	No.	%	No.	%	No.	%
Total PATIENTS with at least one confirmed event	116	46.4%	95	38.0%	76	31.4%
First confirmed event						
WORSENING OF PAH	93	37.2%	72	28.8%	59	24.4%
DEATH	17	6.8%	21	8.4%	16	6.6%
IV/SC PROSTANOID INITIATION	6	2.4%	1	0.4%	1	0.4%
LUNG TRANSPLANTATION	-		1	0.4%	-	

[Source: Table 20 in Sponsor’s CSR, verified by the reviewer]

In the time-to-event analysis, the hazard ratio for the occurrence of a morbidity or mortality event in the macitentan 3 mg group versus placebo was 0.704 (97.5% CLs 0.516, 0.960, logrank  $p = 0.0108$ ). The hazard ratio was 0.547 (97.5% CLs 0.392, 0.762, logrank  $p < 0.0001$ ) in the macitentan 10 mg dose group.

**Figure 1 Kaplan-Meier Curve on the Primary Endpoint Event in SERAPHIN**



### CEC adjudication

A number of patients did not have a second 6MWT measurement to confirm the 15% decrease in 6MWD in order to qualify for a primary endpoint event. Per protocol, “In the situation where despite all efforts to ensure protocol compliance having been undertaken, a second 6-MWT could not be performed as confirmation of clinical worsening of PAH, the CEC will adjudicate on the clinical worsening. If adjudicated as clinical worsening of PAH by the CEC, these events will be included in the primary analysis.” For these patients, CEC received a statement from the investigators justifying the medical reason for the absence of the test and determined to qualify these events for the primary endpoint.

In the CEC report, the deviations from 6MWT guidelines were divided into 4 categories.

Category 1: confirmatory second 6MWT was out of the 2-week time window

Category 2: A new PAH treatment was initiated between the 2 walk tests and therefore the second 6MWT did not confirm at least 15% decrease in 6MWD

Category 3: The 2 confirming 6MWTs were performed on the same day or patient refused to perform a second confirming 6MWT

Category 4: Patients were unable to walk due to severe PAH worsening

As shown in Table 7, the majority of patients who had deviations on the confirmatory second 6MWT belong to Category 4, i.e., they were unable to walk due to severe PAH worsening.

The distribution of the 54 patients in three treatment arms was relatively even. 19 were in placebo group, 18 were in macitentan 3 mg group and 17 in macitentan 10 mg group. A sensitivity analysis similar to the primary analysis using the time to composite endpoint was performed. In the analysis, these 54 patients were censored at the time of event instead of being counted as “other worsening of PAH” events. The conclusion remained unchanged after excluding these events. The hazard ratio of macitentan 10 mg over placebo was 0.51 with 97.5% confidence interval (0.35, 0.74). The hazard ratio of macitentan 3 mg over placebo was 0.68 with 97.5% confidence interval (0.48, 0.96). The p-values based on log-rank test for macitentan 3 mg group and macitentan 10 mg group when compared to placebo were 0.01 and <0.001, respectively. Table 7 lists all 54 patients with deviations in 6MWT.

**Table 7 List of Patients with Deviations on the Confirmatory Second 6MWT**

Center	Patient	Category 1	Category 2	Category 3	Category 4
1001	10960				Yes
1401	14732				Yes
1403	10462	Yes	Yes		
1408	10233	Yes			
1409	10467				Yes
1412	10225				Yes
1501	10858				Yes
2201	14967				Yes
3101	13460				Yes
3104	13236				Yes
3406	12601				Yes
3603	12849				Yes
3603	16229				Yes
3802	14845				Yes
3804	13231			Yes	
3805	13348				Yes
3806	13593	Yes			
3811	13239				Yes
3903	12854				Yes
5105	15225				Yes
5105	15735				Yes
5105	15841				Yes
5107	15853				Yes
5108	15725				Yes
5301	13098		Yes		
5304	13110				Yes
5306	13104				Yes
5306	13117				Yes
5401	13849		Yes		Yes
5501	13737				Yes
5502	10733				Yes
5601	15614				Yes
5702	11229				Yes
5704	13732				Yes
6001	11083				Yes
6001	11098				Yes
6001	11100				Yes
7101	10725				Yes
7102	15081				Yes
7105	13615				Yes
8004	11977				Yes
8005	12222				Yes
8008	12354			Yes	Yes
8009	12084				Yes
8009	12087				Yes
8201	12340				Yes
8203	11359				Yes
8401	11853				Yes
8402	11847	Yes			
8402	12474			Yes	
9103	12093				Yes
9127	11356	Yes			
9127	12118				Yes
9137	12477				Yes

[Source: SERAPHIN Clinical Event Committee Report Appendix 7.3, verified by the reviewer]

Early Discontinuation

371 patients discontinued treatment before the end of the study. Among them, 278 patients had primary events. Among those who did not have any primary events and discontinued treatment early, 37 patients were due to adverse events. 4 patients were due to lack of efficacy and 8 patients had withdrawal consent. 5 patients were loss to follow up. 27 patients withdrew from treatment without stating specific reasons and the rest had various reasons (administrative,

protocol violation, et al). The reviewer performed a time to early discontinuation analysis on all subjects using early discontinuation as the endpoint event to compare macitentan 3 mg and 10 mg with placebo arm. The subjects in macitentan groups stayed significantly longer on treatment than placebo group. The hazard ratio for macitentan 3 mg over placebo was 0.70 with 97.5% confidence interval of (0.53, 0.92). The hazard ratio for macitentan 10 mg over placebo was 0.63 with 97.5% confidence interval of (0.47, 0.84). The p-values from log-rank test on time to discontinuation were 0.004 and <0.001 for macitentan 3 mg and macitentan 10 mg, respectively. Table 8 summarizes the mean and median of treatment duration for the subjects who discontinued treatment early in each treatment group.

**Table 8 Treatment Duration of Subjects with Early Treatment Discontinuation**

	N	Mean	Median
Placebo	147	342.9	278
Macitentan 3 mg	118	396.3	357
Macitentan 10 mg	106	403.6	338

A total of 155 patients discontinued study before the end of study. These include 125 deaths and 14 withdrawal consent. 14 patients were loss to follow up and 2 discontinued study due to administrative reasons.

The study was designed to follow the patients until EOT + 7 days. Ideally, patients should be followed on the primary endpoint events until the end of the study even they may discontinue treatment early during the study. In this trial, there were 93 subjects who did not have any primary events and were censored before the end of the study. These subjects should have been followed longer. 33 of the 93 patients were in placebo arm, 26 were in macitentan 3 mg arm and 34 in macitentan 10 mg arm. The distribution of these subjects are relatively even among three treatment arms.

The sponsor and the reviewer performed a number of sensitivity analyses to assess the impact on the study results from these subjects who discontinued treatment without experiencing a primary endpoint event.

### Sensitivity analyses

A total of 93 patients (33 in placebo group, 26 in low dose group and 34 in high dose group) were censored at the end of treatment instead of the end of the study. One way to test the robustness of the results is to convert these censored patients in high dose one by one to primary events and find out how many more events will be needed in order to change the study conclusion. In this case, at least 18 more events out of the 34 patients in the high dose group were needed to change the p-value of logrank test to 0.005. 26 events out of the 34 patients in the high dose group will be needed to change the p-value to 0.025.

Using investigator reported events with same analysis as primary analysis, the hazard ratio for macitentan 10 mg over placebo was 0.51 with 97.5% confidence interval (0.37, 0.70). The

hazard ratio for macitentan 3 mg over placebo was 0.69 with 97.5% confidence interval (0.51, 0.93). The p-values from log-rank test were <0.001 and 0.004, respectively.

The sponsor also used premature treatment discontinuation as a component of the primary endpoint if patient showed signs of worsening of PAH. To determine which patients were qualified, the sponsor established a list of criteria for identifying patients as showing signs of disease worsening at the end of treatment and performed a blinded review on the patients who discontinued treatment early without a primary endpoint event. The detailed criteria were listed in the statistical analysis plan. This resulted in 12 additional events in placebo arm, 7 additional events in macitentan 3 mg arm and 9 additional events in macitentan 10 mg arm. The hazard ratio for macitentan 10 mg over placebo was 0.56 with 97.5% confidence interval (0.41, 0.77). The hazard ratio for macitentan 3 mg over placebo was 0.69 with 97.5% confidence interval (0.51, 0.92). The p-values from log-rank test were <0.001 and 0.004, respectively.

The sponsor also performed a number of other sensitivity analyses and results appeared consistent.

### Secondary endpoints

The treatment difference in mean change from baseline in 6MWD was 16.8 meters in the macitentan 3 mg group when compared to placebo group and 22.0 meters in the macitentan 10 mg group. The p-values from Wilcoxon rank sum test are 0.008 and 0.012 for the high dose and low dose comparing to placebo, respectively. 114 subjects (52 in placebo, 32 in macitentan 3 mg and 30 in macitentan 10 mg) had missing 6MWD measurements at Month 6. The placebo group has a higher missing rate than the macitentan groups. In order to handle the missing data, the sponsor applied worst value for those who had morbidity/mortality event before month 6 (0 meter for death and 25 percentile value for morbidity events). Detailed imputation rule is explained in *statistical methodologies* section.

The sponsor imputed worst value for 9 deaths and imputed with LOCF for 23 non-death events for the placebo group. The reviewer imputed worst value for 10 deaths and imputed with LOCF for 22 non-death events. This is because one patient (PNO=15725) had a PAH worsening event on (b) (6) and subsequently died on (b) (6). Since the treatment stopped on (b) (6), the death should still be considered as within EOT + 7 days and should be imputed using worst value instead of LOCF.

Table 9. Summary on 6-Minute Walk Test at Month 6

		Placebo	Macitentan 3 mg	Macitentan 10 mg
Baseline	N	249	248	242
	Mean	352.4	364.1	362.6
	STD	110.6	95.5	93.2
	Median	360	378	378
Month 6	N	249	248	242
	Mean	342.9	371.5	375.1
	STD	146.5	124.1	114.7
	Median	365	393.5	390
Total imputed at Month 6 Non Death Event		52 (20.9%)	32 (12.9%)	30 (12.4%)
	Worst value	5	2	0
Death event	Carry forward	22	12	10
	Worst value	10	6	4
No event	baseline carry forward	7	2	10
	Non-baseline carry forward	8	10	6
Treatment Effect	Mean		16.9	22
	STD		96.9	92.6
	97.5% CI		(-2.7, 36.4)	(3.2, 40.8)
	Wilcoxon Rank			
	Sum test p-value		0.012	0.008

The reviewer also performed the same analysis for 6MWD at Month 3, where the data have lower missing rate. The results are consistent with 6MWD at Month 6. This provides some assurance for the robustness of the results in 6MWT despite of the average 15% missing data on 6MWD at Month 6.

**Table 10 Summary on 6-Minute Walk Test at Month 3**

		Placebo	Macitentan 3 mg	Macitentan 10 mg
Total imputed at Month 3		20 (8.0%)	11 (4.4%)	13 (5.4%)
Non Death Event	Worst value	6	3	1
Death event	Worst value	5	3	1
No event	baseline carry forward	9	5	11
Treatment Effect	Mean		17.7	20
	STD		70.7	70.1
	97.5% CI		(3.4, 32.0)	(5.8, 34.2)
	Wilcoxon Rank			
	Sum test p-value		0.003	0.002

19.8% of patients in the macitentan 3 mg group and 22.3% of patients in the macitentan 10 mg group had improvement in WHO functional class compared to 12.9% of patients in the placebo group (Table 10). The p-values based on Fisher's exact test were 0.04 and 0.006 for low dose and high dose macitentan groups, respectively. Table 11 provides details on imputation used for missing WHO function class at Month 6.

**Table 11 Change from Baseline to Month 6 in WHO Functional Class**

	Placebo	Macitentan 3 mg	Macitentan 10 mg
n	249	248	242
Improved	32 12.9%	49 19.8%	54 22.3%
TREATMENT EFFECT:			
Risk Ratio		1.54	1.74
97.5% CLs		0.96, 2.46	1.10, 2.74
p-value Fisher's exact test		0.0395	0.0063

[Source: Table 28 in sponsor's clinical study report, verified by reviewer]

**Table 12 Summary on Imputations for Missing WHO Functional Class**

		Placebo	Macitentan 3 mg	Macitentan 10 mg
WHO FC total imputed at Month 6		49	29	30
Non Death Event	Worst value	2	1	0
	Carry forward	25	12	10
Death event	Worst value	9	6	4
No event	baseline carry forward	5	1	10
	nonbaseline carry forward	8	9	6

Table 12 shows other secondary analyses results. The hazard ratio on time to death due to PAH or hospitalization for PAH up to EOT + 7 days was 0.669 (97.5% CLs 0.462, 0.97, logrank p =

0.0146) in macitentan 3 mg group and 0.50 (97.5% CLs 0.335, 0.747, logrank  $p < 0.001$ ) in macitentan 10 mg group.

21 patients in the macitentan 3 mg group, 14 in the macitentan 10 mg group, and 19 in the placebo group died up to EOT + 7 days. The hazard ratio was 0.971 in the macitentan 3 mg group (97.5% CLs 0.477, 1.976, logrank  $p = 0.92$ ) and 0.638 (97.5% CLs 0.287, 1.418, logrank  $p = 0.20$ ) in the macitentan 10 mg group.

Death of all causes up to EOS was recorded for 47, 34 and 44 patients in the macitentan 3 mg, macitentan 10 mg and placebo groups, respectively. One subject (PNO=5109-16721) in Macitentan 10 mg group died on [REDACTED]<sup>(b) (6)</sup>, which was after the EOS cutoff date (January 30, 2012). This subject therefore was not counted in this analysis. Note that this subject died within EOT + 7 days and was counted in the primary analysis. 9 (3.6%) patients in the macitentan 3 mg, 7 (2.9%) patients in macitentan 10 mg group and 11 (4.4%) patients in placebo group did not have vital status followed to the end of study due to various reasons. The hazard ratio for time to all-cause mortality up to EOS was 1.046 in the macitentan 3 mg group (97.5% CLs 0.653, 1.673, logrank  $p = 0.83$ ) and 0.771 in the macitentan 10 mg group (97.5% CLs 0.464, 1.282, logrank  $p = 0.25$ ).

**Table 13 Summary on Other Secondary Analyses**

		placebo (N=249)	macitentan 3 mg (N=248)	macitentan 10 mg (N=242)
Time to PAH death or PAH hosp	# of events	84	65	50
	hazard ratio p-value		0.67 (0.46, 0.97) 0.015	0.5 (0.34, 0.75) <0.001
Time to all cause death up to EOT+7	# of events	19	21	14
	hazard ratio p-value		0.97 (0.48, 1.98) 0.92	0.64 (0.29, 1.42) 0.20
Time to all cause death up to EOS	# of events	44	47	34
	hazard ratio p-value		1.05 (0.65, 1.67) 0.83	0.77 (0.46, 1.28) 0.25

## 4. FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

### 4.1 Gender, Race, Age, and Geographic Region

The subgroup analysis by region (**Figure 2** and **Figure 3**) showed that the treatment effect of macitentan groups was trending in the wrong direction in US. US had an extreme low primary event rate in the placebo group (17% versus an average of 49% in other regions), while the event rate in the treatment group seemed comparable in US and OUS. The change from baseline in 6MWD at Month 6 also trended in the wrong direction for US (Table 14). However, caution needs to be taken in interpreting this finding due to very small sample size in US.

Table 14 Summary on Change from Baseline in 6MWD in US and OUS

	Placebo			Macitentan 3 mg			Macitentan 10 mg		
	N	Mean	STD	N	Mean	STD	N	Mean	STD
US	23	5.7	57.8	25	-22.7	92.7	19	-34.5	105
OUS	226	-11	103.9	223	10.7	92.8	223	16.5	80.5

The treatment effect in subjects who were on PAH background therapy appeared to be numerically less than the subjects who had no PAH background therapy.

Figure 2 Forest Plot on Subgroup Analyses (Low Dose versus Placebo)

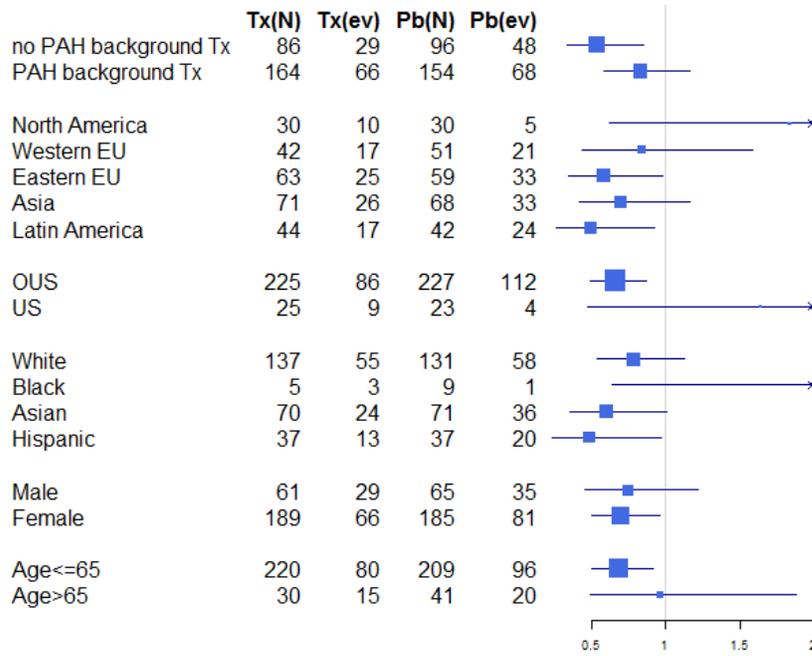
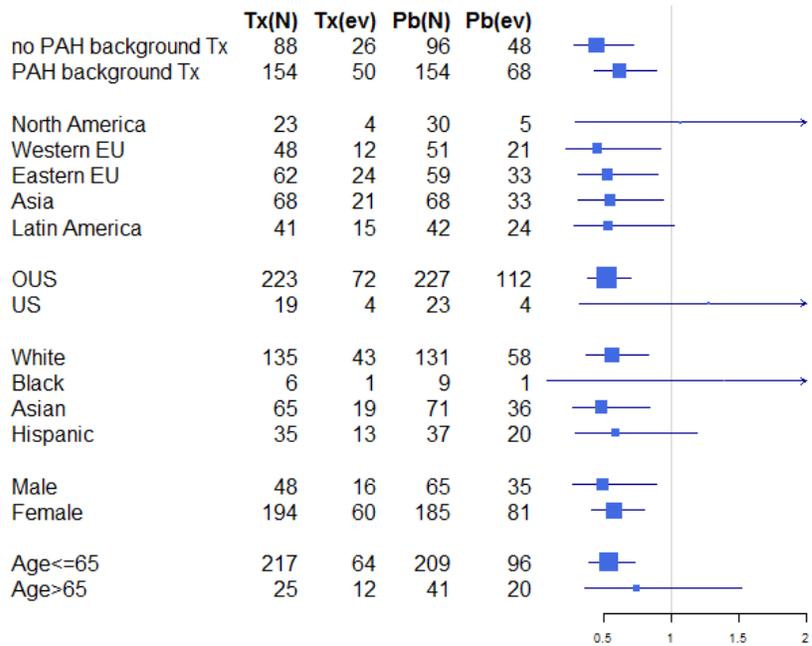


Figure 3 Forest Plot on Subgroup Analyses (High Dose versus Placebo)



## 4.2 Other Special/Subgroup Populations

No other subgroups were analyzed.

# 5. SUMMARY AND CONCLUSIONS

## 5.1 Statistical Issues and Collective Evidence

The single phase III trial showed highly significant results in the primary endpoint. The primary endpoint of the study was a composite morbidity/mortality endpoint with multiple components, including death, lung transplantation, initiation of prostanoids, atrial septostomy and other worsening of PAH. The primary endpoint was driven by the single component “other worsening of PAH” with no effect shown for mortality. “Other worsening of PAH” was defined with three criteria (15% decrease in 6MWD, worsening of PAH symptoms in terms of WHO FC or right heart failure, need for new PAH treatment). 54 patients with CEC-adjudicated “other worsening of PAH” events had some deviation in measuring the second confirmatory 6MWD. Most of these subjects did not take the second 6MWT and the decision of qualifying these events were based on written justifications from the investigators. However, the distribution of these subjects in three treatment arms was relatively even (19 in placebo group, 18 in macitentan 3 mg group and 17 in macitentan 10 mg group). Excluding these events did not affect the study results much. This provides some assurance for the analysis results.

The subjects were followed for the primary events until the end of treatment + 7 days instead of the end of study. As a result, 93 patients discontinued treatment early and censored at EOT + 7 days without any primary endpoint events. The sponsor and the reviewer performed a number of sensitivity analyses to assess the impact on the study results from these patients who discontinued treatment without experiencing a primary endpoint event. To test the robustness of the results, the reviewer converted these censored patients in high dose to primary events one by one. At least 18 more events out of the 34 patients in the high dose group were needed to change the p-value of logrank test to 0.005. Other sensitivity analyses showed consistent results overall.

To address patient early discontinuation, a time to early discontinuation analysis on all patients was performed using early discontinuation as the endpoint event to compare macitentan groups with placebo arm. The patients in macitentan groups stayed significantly longer on treatment than placebo group.

The subgroup analysis by region showed that the treatment effect of macitentan groups was trending in the wrong direction in US. US had an extreme low primary event rate in the placebo group (17% versus an average of 49% in other regions), while the event rate in the treatment group seemed comparable in US and OUS. The 6MWD at Month 6 also trended in the wrong direction for US. Caution needs to be taken in interpreting this finding due to very small sample size in US.

On average, about 15% patients had missing 6MWD measurements at Month 6. The percentage of missing was higher in placebo group (21%) and lower in macitentan groups (13% in low dose group and 12% in high dose group). The patients in macitentan 10 mg group and macitentan 3 mg group had 22 meter and 17 meter improvement in 6-minute walk distance at Month 6, respectively, when compared to placebo. The reviewer also examined the 6MWD at Month 3, which had less missing data (6%). The results in 6MWD at Month 3 were consistent with the results in 6MWD at Month 6.

## **5.2 Conclusions and Recommendations**

The single phase III trial showed highly significant results in the primary endpoint. However, the primary endpoint was driven by a single component “other worsening of PAH” with no effect shown for mortality. Some subjects with “other worsening of PAH” events had deviation in measuring the second confirmatory 6MWD. Most of these subjects did not take the second 6MWT. Excluding these events did not change the study conclusion. Subjects were followed for the primary events until the end of treatment + 7 days instead of the end of study. As a result, 93 subjects discontinued treatment early and censored without any primary endpoint events. Sensitivity analyses showed that study results appeared to be robust. The subjects in macitentan 10 mg group had 22 meter improvement in 6-minute walk distance at Month 6 when compared to placebo. The results of 6-minute walk test at Month 3 were consistent with less missing data. The subgroup analysis showed that the treatment effect of macitentan groups was trending in the wrong direction in US. But caution needs to be taken in interpreting this finding due to very small sample size in US. Overall, the results in SERAPHIN trial seem to support the efficacy of macitentan 10 mg.

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/s/  
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JIALU ZHANG  
06/18/2013

HSIEN MING J HUNG  
06/18/2013



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

## Statistical Review and Evaluation

### CARCINOGENICITY STUDIES

**IND/NDA Number:** NDA 204-410

**Drug Name:** ACT-064992 (Opsumit)

**Indication(s):** 104 Week Rat and Mouse Carcinogenicity Studies

**Applicant:** **Sponsor:** Actelion Pharmaceuticals Ltd  
Gewerbstrasse 16, 4123 Allschwil, Switzerland

**Test facility:** [REDACTED] (b) (4)

**Documents Reviewed:** Electronic submission submitted on October 19, 2012  
Electronic data submitted on October 19, 2012

**Review Priority:** Standard

**Biometrics Division:** Division of Biometrics -6

**Statistical Reviewer:** Mohammad Atiar Rahman, Ph.D.

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**Medical Division:** Division of Cardiovascular and Renal Products

**Reviewing Pharmacologist:** William T. Link, Ph.D.

**Project Manager:** Dan Brum

**Keywords:** Carcinogenicity, Dose response

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## 1. Background

In this submission the sponsor included reports of two animal carcinogenicity studies, one in rats and one in mice. These studies were intended to assess the carcinogenic potential of ACT-064992 (Opsumit) when administered orally by gavage once daily at appropriate drug levels for 104 weeks. Results of this review have been discussed with the reviewing pharmacologist Dr. Link.

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

## 2. Rat Study

Two separate experiments were conducted, one in male and one in female rats. In each of these two experiments there were three treated groups and two identical vehicle control groups. Two hundred and fifty five HanRcc: SPF Wister rats of each sex were assigned randomly to the treated and vehicle control groups in equal size of 51 rats per group. The dose levels for treated groups were 10, 50, or 250 mg/kg/day for males and 10, 50/25, or 250/50 mg/kg/day for females. Due to high morbidity conditions, there was an interim sacrifice of animal prior to Week 48. At Week 48 the dosing was stopped and restarted after 2 weeks of non-dosing period at the original dose levels for male rats and at dose levels of 10, 25, or 50 mg/kg/day for female rats. In this review these dose groups would be referred to as the low, medium, and high dose groups, respectively. The two control groups were given the vehicle (0.5% (w/v) methylcellulose) daily by oral gavage.

During the administration period all rats were observed twice daily, at the beginning and at the end of each working day, during the entire study period for mortality and morbidity. All rats were observed once daily during acclimatization, twice daily during the first two treatment weeks and once daily thereafter for clinical signs. The rats were palpated regularly for the appearance of masses during the clinical observations.

Body weights of all rats were measured once during acclimatization, on treatment Day 1, weekly up to start of Week 18 (day 120), every four weeks thereafter and before necropsy.

### 2.1. Sponsor's analyses

#### 2.1.1. Survival analysis

Survival function of each treatment group was estimated using the Kaplan-Meier product limit method and was presented graphically. Data were analyzed using the Cox regression method.

**Sponsor's findings:** The sponsor analysis showed 23%, 37%, 45% and 35% mortalities in male rats, and 23%, 31%, 35%, and 59% mortalities in female rats in combined control, low, medium, and high dose groups, respectively.

The sponsor concluded that there was no dose-related effect on survival in any dose levels of male rats, and in the low and medium dose levels of female rats. There was a high mortality in female high dose groups. The sponsor commented that for the increased mortality in female high dose group no overt pathological changes could be identified. There was no single or preponderant cause of morbidity/mortality that could be attributed to the effect of test item.

### 2.1.2. Tumor data analysis

The tumor incidence data were analyzed using the methods outlined in the paper of Peto et al. (1980) for positive dose response relationships and the Fisher exact test for pairwise comparisons. The sponsor conducted three sets of analysis (1) combined control versus the treated groups, (2) the first control groups versus the treated groups, and (3) the second control group versus the treated groups. The results are commented if p-values were below of 0.05.

**Adjustment for multiple testing:** The sponsor adjusted the multiple dose response relationship tests using the recommendations of Lin and Rahman (1998). For dose response relationship tests in tumor data analysis, Lin and Rahman recommended the use of  $\alpha=0.005$  for common tumors and test  $\alpha=0.025$  for rare tumors for a study with two species, and  $\alpha=0.01$  for common tumors and test  $\alpha=0.05$  for rare tumors for a study with one species. Common tumors were defined as tumors with more than 1% background rate and rare otherwise. These values of test levels were recommended in order to keep an overall false positive rate of about 10%.

**Sponsor's findings:** The sponsor's analyses did not show statistically significant dose response relationship among the treatment groups in any of the observed tumors. Pairwise comparisons also did not show increased incidence in any of the observed tumors.

## 2.2. Reviewer's analyses

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

As mentioned earlier that the submitted rat study had two identical vehicle control groups. For studies with two identical controls, the FDA statistical guidance for carcinogenicity data analysis suggests to analyze the data combining the two control groups. Following the guidance suggestion, this reviewer analyzed both the mortality and tumor data using the combined control.

### 2.2.1. Survival analysis

The survival distributions of rats in all five treatment groups were estimated using the Kaplan-Meier product limit method. For combined control, low, medium, and high dose groups, the dose response relationship was tested using the likelihood ratio test and the homogeneity of survival distributions was tested using the log-rank test. The Kaplan-Meier curves for survival rates are given in Figures 1A and 1B in the appendix for male and female rats, respectively. The intercurrent mortality data are given in Tables 1A and 1B in the appendix for male and female rats, respectively. Results of the tests for dose response relationship and homogeneity of survivals, are given in Tables 2A and 2B in the appendix for male and female rats, respectively.

**Reviewer's findings:** This reviewer's analysis showed 25%, 20%, 35%, 45%, and 35% mortality of male rats and 24%, 22%, 32%, 33%, and 59% mortality of female rats in control 1, control 2, low, medium, and high dose groups, respectively. The tests showed statistically significant dose response relationship in mortality across combined control and treated groups in female rats. The pairwise comparisons showed statistically significant increased mortality in the male rat medium dose group and female rat high dose group compared to their respective combined control.

**Reviewer's comment:** *In the male low dose group, the sponsor's calculation showed 37% mortality while this reviewer's calculation*

*showed 35%. Also in the female medium dose group, the sponsor's calculation showed 35% mortality while this reviewer's calculation showed 33%. These differences were due to the facts that one male rat (#149) from low dose group, and one female rat (#488) from medium dose group died due to natural causes during the terminal sacrifice weeks. The sponsor counted them with the naturally dead rats, while this reviewer counted them with the terminally sacrificed rats.*

### 2.2.2. Tumor data analysis

The tumor data were analyzed for dose response relationships and pairwise comparisons of combined control group with each of the treated groups. Both the dose response relationship tests and pairwise comparisons were performed using the Poly-k method described in the paper of Bailer and Portier (1988) and Bieler and Williams (1993). In this method an animal that lives the full study period ( $w_{\max}$ ) or dies before the terminal sacrifice but develops the tumor type being tested gets a score of  $s_h = 1$ . An animal that dies at week  $w_h$  without a tumor

before the end of the study gets a score of  $s_h = \left( \frac{w_h}{w_{\max}} \right)^k < 1$ . The adjusted group size is defined as  $\sum s_h$ . As an

interpretation, an animal with score  $s_h = 1$  can be considered as a whole animal while an animal with score  $s_h < 1$  can be considered as a partial animal. The adjusted group size  $\sum s_h$  is equal to N (the original group size) if all animals live up to the end of the study or if each animal that dies before the terminal sacrifice develops at least one tumor, otherwise the adjusted group size is less than N. These adjusted group sizes are then used for the dose response relationship (or the pairwise) tests using the Cochran-Armitage test. One critical point for Poly-k test is the choice of the appropriate value of k, which depends on the tumor incidence pattern with the increased dose. For long term 104 week standard rat and mouse studies, a value of k=3 is suggested in the literature. Hence, this reviewer used k=3 for the analysis of this data. For the calculation of p-values the exact permutation method was used. The tumor rates and the p-values of the tested tumor types are listed in Tables 3A and 3B in the appendix for male and female rats, respectively.

**Multiple testing adjustment:** For the adjustment of multiple testing of dose response relationship, the FDA guidance for the carcinogenicity study design and data analysis suggests the use of test levels  $\alpha=0.005$  for common tumors and  $\alpha=0.025$  for rare tumors for a submission with two species, and a significance level  $\alpha=0.01$  for common tumors and  $\alpha=0.05$  for rare tumors for a submission with one species in order to keep the false-positive rate at the nominal level of approximately 10%. A rare tumor is defined as one in which the published spontaneous tumor rate is less than 1%. For multiple pairwise comparisons of treated group with control the FDA guidance suggested the use of test levels  $\alpha=0.01$  for common tumors and  $\alpha=0.05$  for rare tumors, in order to keep the false-positive rate at the nominal level of approximately 10% for both submissions with two or one species.

It should be noted that the FDA guidance for multiple testing for dose response relationship is based on a publication by Lin and Rahman (1998). In this work the authors investigated the use of this rule for Peto analysis. However, in a later work Lin and Rahman (2008) showed that this rule for multiple testing for dose response relationship is also suitable for Poly-K tests.

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

**Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pairwise Comparisons of Treated Groups and Combined Control in Rats**

Sex	Organ Name	Tumor Name	Com C <sup>#</sup>	Low	Med	High	P_Val ue			
							Dose Resp	Com C vs. L	Com C vs. M	Com C vs. H
Male	SKIN/SUBCUTIS	Squamous cell carcinoma	0	1	3	1	0.2698	0.3212	0.0286*	0.3261
Female	UTERUS	Stromal polyp	7	4	3	7	0.0302	0.5095	0.6673	0.0387

# Com C: Combined Control

Based on the criteria of adjustment for multiple testing discussed above, none of the observed tumors was considered to have statistically significant dose response relationship in either sex. The pairwise comparison was considered to be statistically significant for the increased incidence of squamous cell carcinoma in skin/subcutis in the male rat medium dose group compared to the combined control.

**3. Mouse Study**

Two separate experiments were conducted, one in male and one in female mice. In each of these two experiments there were four treated groups and one vehicle control group. Three hundred SPF-bred B6C3F1 mice of each sex were assigned randomly to the treated and vehicle control groups in equal size of 60 mice per group. The dose levels for treated groups were 5, 30, 100 and 400 for both sexes. In this review these dose groups were referred to as the low, medium, mid-hi, and high dose groups, respectively. Animals in the vehicle control group received the vehicle (0.5% (w/v) methylcellulose) by gavage. As mentioned earlier, the mouse study was scheduled to be of 104 week long. However, due to excessive morbidity/mortality, the female high dose group was terminated on Week 79. Due to their relatively young age, the sponsor assumed that these animals were unlikely to contribute meaningfully to the oncogenic assessment. The microscopic examination of organs of all high dose group female mice was suspended, except for few mice in context to their target organs.

All mice were observed twice daily, at the beginning and at the end of each working day during the entire study period. Clinical signs, including palpation for tissue masses, were observed once daily during acclimatization, twice daily during the first two treatment weeks, and once daily thereafter.

Body Weights were measured weekly during acclimatization week 1 and 3, twice during acclimatization week 2, on treatment day 1, weekly up to week 17, every four weeks thereafter and before necropsy.

**3.1. Sponsor's analyses**

**3.1.1. Survival analysis**

The sponsor used similar methodologies to analyze the mouse survival data as they used to analyze the rat survival data.

**Sponsor’s findings:** The sponsor analysis showed 7%, 15%, 7%, 13%, and 15% mortalities in male mice, and 32%, 32%, 25%, 35%, and 58% mortalities in female mice in control, low, medium, mid-hi, and high dose groups, respectively.

The sponsor concluded that the female high dose group showed significant high morbidity/mortality. The remaining treatment groups did not show significant differences in mortality compared to their respective control.

### 3.1.2. Tumor data analysis

The sponsor used similar methodologies to analyze the mouse tumor data as they used to analyze the rat tumor data.

**Adjustment for multiple testing:** The sponsor used similar procedure to adjust the multiple testing in mouse tumor data analysis as they used to adjust the multiple testing in rat data analysis.

**Sponsor's findings:** The sponsor's analyses did not show statistically significant dose response relationship among the treatment groups in any of the observed tumors. Pairwise comparisons also did not show increased incidence in any of the observed tumors.

## 3.2. Reviewer's analyses

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

For the analysis of both the survival data and the tumor data this reviewer used similar methodologies as he used for the analyses of the rat survival and tumor data.

As mentioned earlier, all mice in the female high dose group were terminated at Week 79. Therefore, the mice in the female high dose group might not have been exposed to the study drug long enough for sufficient challenge to show its possible carcinogenic potential. Hence, it is reasonable to analyze the female mouse data by dropping the high dose group. The submitted mouse data had tumorigenicity information of control through high dose for both sexes. The female high dose had data of only four mice in context to some target organs. In consideration to the above discussion, this reviewer analyzed the female mouse tumor data by dropping the high dose group. However, since tumor data from some female high dose mice were included in the submitted data, this reviewer performed an exploratory analysis including the data from the high dose group.

### 3.2.1. Survival analysis

The Kaplan-Meier curves for survival rates of all treatment groups are given in Figures 2A and 2B in the appendix for male and female mice, respectively. The intercurrent mortality data of all treatment groups are given in Tables 4A and 4B in the appendix for male and female mice, respectively. Results of the tests for dose response relationship and homogeneity of survivals for control, low, medium, mid-hi, and high dose groups are given in Tables 5A and 5B in the appendix for male and female mice, respectively.

**Reviewer's findings:** This reviewer's analysis showed 7%, 12%, 5%, 13%, and 13% mortalities in male mice, and 28%, 32%, 23%, 30%, and 55% mortality in female mice in control, low, medium, mid-hi, and high dose groups, respectively. The tests showed statistically significant dose response relationship in mortality across the treatment groups in female mice. The pairwise comparison show statistically significant decreased mortality in male mice medium dose group compared to their control. The pairwise comparisons also showed statistically significant increased mortality in female mice high dose group compared to their control.

**Reviewer’s comment:** *There are several discrepancies between the mortality calculations of the sponsor and this reviewer. These discrepancies were due to the fact that the following mice died due to natural causes during the end of the study terminal weeks or interim sacrifice at Week 79. The sponsor counted these mice with the naturally dead animals, while this reviewer counted them with the terminally sacrificed mice.*

**Animals Died Due to Natural Causes During End of Study Termination Weeks or Interim Sacrifice Weeks**

	<i>Veh. Control</i>	<i>Low</i>	<i>Medium</i>	<i>Mid-Hi</i>	<i>High</i>
<i>Male</i>		#93, #137	#227		#373
<i>Female</i>	#461, #472		#624	#687, #702, #725	#760, #761

**3.2.2. Tumor data analysis**

The tumor rates and the p-values of the tested tumor types for male mice are given in Tables 6A in the appendix, for female mice excluding the high dose group are given in Tables 6B in the appendix, and for female mice including the high dose group are given in Tables 6C in the appendix.

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

**Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pairwise Comparisons of Treated Groups and Vehicle Control in Mice**

Sex	Organ Name	Tumor Name	Dose					P_Val ue				
			Veh C N=60	Low N=60	Med N=60	Mid-Hi N=60	High N=60	Dose Resp	VC vs L	VC vs M	VC vs MH	VC vs H
Female	DUODENUM+ILEUM	Adenoma	0	0	0	1	1	0.0459	.	.	0.4952	0.2740

Veh C: Vehicle Control

Based on the criteria of adjustment for multiple testing discussed in the rat data analysis section, none of the observed tumor types was considered to have statistically significant dose response relationship in either sex. The pairwise comparisons also did not show statistically significant increased incidence in any of the observed tumor types in any treated groups compared to their respective control in either sex.

**4. Evaluation of the validity of design of rat and mouse studies**

As has been noted, the tumor data analyses from both rat and mouse studies did not show statistically significant dose-response relationship in any of the observed tumor types. However, before drawing any conclusion regarding the carcinogenic or non-carcinogenic potential of the study drug in rats and mice, it is important to look into the following two issues, as have been pointed out in the paper by Haseman (1984).

- (i) Were enough animals exposed, for a sustained amount of time, to the risk of late developing tumors?
- (ii) Were dose levels high enough to pose a reasonable tumor challenge to the animals?

There is no consensus among experts regarding the number of animals and length of time at risk, although most carcinogenicity studies are designed to run for two years with about fifty to sixty animals per treatment group. The

following are some rules of thumb regarding these two issues as suggested by experts in this field.

Haseman (1985) has done an investigation on the first issue. He gathered data from 21 studies using Fischer 344 rats and B6C3F1 mice conducted at the National Toxicology Program (NTP). It was found that, on the average, approximately 50% of the animals in the high dose group survived the two-year study period. Also, in a personal communication with Dr. Karl Lin of Division of Biometrics-6, Haseman suggested that, as a rule of thumb, a 50% survival of 50 initial animals or 20 to 30 animals still alive in the high dose group, between weeks 80-90, would be considered as a sufficient number and adequate exposure. In addition Chu, Cueto and Ward (1981), suggested that "to be considered adequate, an experiment that has not shown a chemical to be carcinogenic should have groups of animals with greater than 50% survival at one-year."

It appears, from these three sources that the proportions of survival at 52 weeks, 80-90 weeks, and two years are of interest in determining the adequacy of exposure and number of animals at risk.

Regarding the question of adequate dose levels, it is generally accepted that the high dose should be close to the maximum tolerated dose (MTD). In the paper of Chu, Cueto and Ward (1981), the following criteria are mentioned for dose adequacy. A high dose is considered as close to MTD if any of the criteria is met.

- (i) "A dose is considered adequate if there is a detectable loss in weight gain of up to 10% in a dosed group relative to the controls."
- (ii) "The administered dose is also considered an MTD if dosed animals exhibit clinical signs or severe histopathologic toxic effects attributed to the chemical."
- (iii) "In addition, doses are considered adequate if the dosed animals show a slight increased mortality compared to the controls."

We will now investigate the validity of the ACT-064992 rat and mouse carcinogenicity study, in the light of the above guidelines.

#### 4.1. Rat Study

The following is the summary of survival data of rats in the high dose groups:

##### Percentage of Survival in the High Dose Group at the End of Weeks 52, 78, and 91

	____ Percentage of survival ____		
	End of 52 weeks	End of 78 weeks	End of 91 weeks
Male	98%	96%	82%
Female	75%	67%	55%

Based on the survival criterion Haseman proposed, it may be concluded that enough rats were exposed to the high dose for a sufficient amount of time in both sexes.

The following table shows the percent difference in mean body weight gain in rats from the concurrent combined control, defined as

$$\text{Percent difference} = \frac{(\text{Final BW} - \text{Baseline BW})_{\text{Treated}} - (\text{Final BW} - \text{Baseline BW})_{\text{Control}}}{(\text{Final BW} - \text{Baseline BW})_{\text{Control}}} \times 100$$

**Percent Difference in Mean body Weight Gain from Combined Controls**

Male			Female		
Low	Medium	High	Low	Medium	High
0.56	-5.22	-18.83	-8.44	-34.45	-54.29

Source: "Body weights – summary main study animals" of Sponsor's report (Part of Table 8)

Therefore, relative to combined control the male rats in high dose group had more than 18% and the female rats had more than 54% decrements in their body weight gains.

The mortality rates at the end of the experiment were as follows:

**Mortality Rates at the End of the Experiment**

	Combined Control			
	Low	Medium	High	
Male	23%	35%	45%	35%
Female	23%	31%	33%	58%

This shows that the mortality rates in the male rats high dose group is 10% and that in female rats is 33% higher than their respective combined control.

Thus, from the mortality and the body weight gain data it can be concluded that the used high dose level might have reached or exceeded the MTD in both sexes. From similar considerations, the medium dose also seems to be close to MTD. For a final determination of the adequacy of the doses used, other clinical signs and histopathological toxic effects must be considered.

**4.2. Mouse Study**

The following is the summary of survival data of mice in the high dose groups:

**Percentage of Survival in the High Dose Group at the End of Weeks 52, 78, and 91**

	Percentage of survival		
	End of 52 weeks	End of 78 weeks	End of 91 weeks
Male	98%	92%	87%
Female	93%	45%	0

Based on the survival criterion Haseman proposed, it may be concluded that enough male mice were exposed to the high dose for a sufficient amount of time. Not enough female mice might have been exposed to the high dose for a sufficient amount of time.

The following table shows the percent difference in mean body weight gain in mice from the concurrent control,

#### Percent Difference in Mean body Weight Gain from Controls

Male				Female			
Low	Medium	Mid-Hi	High	Low	Medium	Mid-Hi	High
-0.70	-9.47	-9.47	-29.12	-11.03	-14.71	-37.50	-50.00

Source: Source: "Body weights – summary main study animals" of Sponsor's report (Part of Table 8)

Therefore, relative to control the high dose male mice had more than 29% and the female mice had 50% decrement in their body weight gain.

The mortality rates at the end of the experiment were as follows:

#### Mortality Rates at the End of the Experiment

	Vehicle Control	Low	Medium	Mid-Hi	High
Male	7%	12%	5%	13%	13%
Female	28%	32%	23%	30%	100%

This shows that the mortality rate of in the male mice high dose group is 6% higher than the control. None of the female mice lived up to the end of end of the study.

Thus, from the body weight gain and mortality data it can be concluded that the used high dose level might have reached or exceeded the MTD in both sexes. From similar considerations, the mid-hi dose also seems to be close to MTD.

## 5. Summary

In this submission the sponsor included reports of two animal carcinogenicity studies, one in rats and one in mice. These studies were intended to assess the carcinogenic potential of ACT-064992 (Opsumit) when administered orally by gavage once daily at appropriate drug levels for 104 weeks.

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

**Rat Study:** Two separate experiments were conducted, one in male and one in female rats. In each of these two experiments there were three treated groups and two identical vehicle control groups. Two hundred and fifty five HanRcc: SPF Wister rats of each sex were assigned randomly to the treated and vehicle control groups in equal size of 51 rats per group. The dose levels for treated groups were 10, 50, or 250 mg/kg/day for males and 10, 50/25, or 250/50 mg/kg/day for females. Due to high morbidity conditions there was an interim sacrifice of animal prior to Week 48. At Week 48 the dosing was stopped and restarted after 2 weeks of non-dosing period at the original dose levels for male rats and at dose levels of 10, 25, or 50 mg/kg/day for female rats. The two control groups were given the vehicle (0.5% (w/v) methylcellulose) daily by oral gavage.

During the administration period all rats were observed twice daily at the beginning and at the end of each working day during the entire study period for mortality and morbidity. All rats were observed once daily during acclimatization, twice daily during the first two treatment weeks and once daily thereafter for clinical signs. The rats were palpated regularly for the appearance of masses during the clinical observations.

Body weights of all rats were measured once during acclimatization, on treatment Day 1, weekly up to start of Week 18 (day 120), every four weeks thereafter and before necropsy.

The tests showed statistically significant dose response relationship in mortality across combined control and treated groups in female rats. The pairwise comparisons showed statistically significant increased mortality in the male rat medium dose group and female rat high dose group compared to their respective combined control.

The tests did not show statistically significant dose response relationship in any of the observed tumors in either sex. The pairwise comparison was considered to be statistically significant for the increased incidence of squamous cell carcinoma in skin/subcutis in the male rat medium dose group compared to the combined control.

**Mouse Study:** Two separate experiments were conducted, one in male and one in female mice. In each of these two experiments there were four treated groups and one vehicle control group. Three hundred SPF-bred B6C3F1 mice of each sex were assigned randomly to the treated and vehicle control groups in equal size of 60 mice per group. The dose levels for treated groups were 5, 30, 100 and 400 for both sexes. Animals in the vehicle control group received the vehicle (0.5% (w/v) methylcellulose) by gavage. The mouse study was scheduled to be of 104 week long. However, due to excessive morbidity/mortality, the female high dose group was terminated on Week 79. Due to their relatively young age, the sponsor assumed that these animals were unlikely to contribute meaningfully to the oncogenic assessment. Therefore, their microscopic examination was suspended, except for few mice in context to their target organs.

All mice were observed twice daily, at the beginning and at the end of each working day during the entire study period. Clinical signs, including palpation for tissue masses, were observed once daily during acclimatization, twice daily during the first two treatment weeks, and once daily thereafter.

Body Weights were measured weekly during acclimatization weeks 1 and 3, twice during acclimatization week 2, on treatment day 1, weekly up to week 17, every four weeks thereafter and before necropsy.

The tests showed statistically significant dose response relationship in mortality across the treatment groups in female mice. The pairwise comparison show statistically significant decreased mortality in male mice medium dose group compared to their control. The pairwise comparisons also showed statistically significant increased mortality in female mice high dose group compared to their control.

The tests did not show statistically significant dose response relationship in the incidence of any of the observed tumor types in either sex. The pairwise comparisons also did not show statistically significant increased incidence in any of the observed tumor types in any treated groups compared to their respective control in either sex.

**Evaluation of the study design:** The mortality and body weight gain data indicate that the used high dose level might have reached or exceeded the MTD in both sexes of rats and mice. For a final determination of the adequacy of the doses used, other clinical signs and histopathological toxic effects must be considered.

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6. Appendix

**Table 1A: Intercurrent Mortality Rate  
Male Rats**

Week	Control 1		Control 2		10 mg		50 mg		250 mg	
	No. of Death	Cum. %								
fff										
0 - 52	1	1.96	1	1.96	1	1.96	1	1.96	1	1.96
53 - 78	2	5.88	1	3.92	2	5.88	6	13.73	1	3.92
79 - 91	4	13.73	6	15.69	8	21.57	4	21.57	7	17.65
92 - 104	6	25.49	2	19.61	7	35.29	12	45.10	9	35.29
Ter. Sac.	38	74.51	41	80.39	33	64.71	28	54.90	33	64.71
-----										
Total	N=51									

\* Cum. %: Cumulative percentage

**Table 1B: Intercurrent Mortality Rate  
Female Rats**

Week	Control 1		Control 2		10 mg		50/25 mg		250/50 mg	
	No. of Death	Cum. %								
fff										
0 - 52	.	.	1	1.96	1	1.96	3	5.88	13	25.49
53 - 78	4	7.84	2	5.88	2	5.88	3	11.76	4	33.33
79 - 91	4	15.69	2	9.80	6	17.65	5	21.57	6	45.10
92 - 104	4	23.53	6	21.57	7	31.37	6	33.33	7	58.82
Ter. Sac.	39	76.47	40	78.43	35	68.63	34	66.67	21	41.18
-----										
Total	N=51									

\* Cum. %: Cumulative percentage

**Table 2A: Intercurrent Mortality Comparison  
Male Rats**

Test	Statistic	P_Value#
fff		
Dose-Response	Likelihood Ratio	0.6580
Homogeneity	Log-Rank	0.0341

#P-Values were calculated using data from Combined Control, Low, Medium, and High dose groups

**Table 2B: Intercurrent Mortality Comparison  
Female Rats**

Test	Statistic	P_Value#
fff		
Dose-Response	Likelihood Ratio	<.0001
Homogeneity	Log-Rank	<.0001

#P-Values were calculated using data from Combined Control, Low, Medium, and High dose groups

**Table 3A: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons Male Rats**

Organ Name	Tumor Name	0mg Com C N=102	10mg Low N=51	50mg Med N=51	250mg High N=51	P_Val ue Dose Resp	P_Val ue Com C vs. L	P_Val ue Com C vs. M	P_Val ue Com C vs. H
ADRENAL GLANDS	Hemangi osarcoma	0	0	0	1	0.2018	.	.	0.3261
ADRENAL MEDULLA	Benign medullary tumor	3	2	0	3	0.1778	0.5068	1.0000	0.2929
	Malignant medullary tumor	1	0	0	0	1.0000	1.0000	1.0000	1.0000
BODY CAVITIES	Hemangi oma	0	0	0	1	0.1982	.	.	0.3212
	Hemangi osarcoma	0	0	0	1	0.1982	.	.	0.3212
	Malignant hi bernoma	0	0	1	0	0.3901	.	0.3162	.
	Osteosarcoma	0	0	1	0	0.3874	.	0.3111	.
BONE	Osteochondroma, in the nasal cavi	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	Osteoma	1	1	0	0	0.8256	0.5340	1.0000	1.0000
BRAIN	Oligodendrogl ioma	3	0	1	2	0.2597	1.0000	0.7799	0.5175
	Pi neal gland tumor	0	1	0	0	0.5830	0.3212	.	.
DUODENUM	Adenocarci noma, sci rrhous	1	0	0	0	1.0000	1.0000	1.0000	1.0000
EPI DI DYMI DES	Beni gn fi brous hi sti ocytoma	0	1	0	0	0.5811	0.3162	.	.
	Fi brosarcoma	0	0	1	0	0.3874	.	0.3111	.
HEART INCL. AUR	Mal i gnant mesothel ioma	0	1	0	0	0.5830	0.3212	.	.
	Mal i gnant schwannoma	0	1	0	0	0.5830	0.3212	.	.
JEJUNUM W/PEYER	Adenocarci noma, mucinous	1	0	0	1	0.3578	1.0000	1.0000	0.5408
KIDNEYS	Li poma	1	1	0	0	0.8272	0.5408	1.0000	1.0000
LIVER	Hepatocel lular adenoma	1	0	2	1	0.2341	1.0000	0.2283	0.5408
	Hepatocel lular carci noma	1	0	1	0	0.6258	1.0000	0.5270	1.0000
LUNG	Metastasi s of carci noma, si te of	0	0	0	1	0.1982	.	.	0.3212
LYMPH NODE MESE	Hemangi oma	0	0	1	0	0.3874	.	0.3111	.
	Lymphangi osarcoma	1	0	0	0	1.0000	1.0000	1.0000	1.0000
LYMPH NODES	Lymphangi oma	0	0	1	0	0.3874	.	0.3111	.
MAMMARY GLAND A	Adenocarci noma	0	1	0	0	0.5811	0.3162	.	.
NASAL CAVITY	Squamous cell carci noma	1	3	0	0	0.8736	0.0974	1.0000	1.0000
ORAL CAVITY	Squamous cell papi loma, seen in	1	0	0	0	1.0000	1.0000	1.0000	1.0000
PANCREAS	I slet cell adenoma	4	2	2	2	0.4610	0.6290	0.6079	0.6290
	I slet cell carci noma	3	0	1	2	0.2571	1.0000	0.7794	0.5170
	I slet_cell _adnoma+carci noma	7	2	3	4	0.2948	0.8481	0.6542	0.4947

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Com C: Combined Control

**Table 3A: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons Male Rats**

Organ Name	Tumor Name	0mg Com C N=102	10mg Low N=51	50mg Med N=51	250mg High N=51	P_Val ue Dose Resp	P_Val ue Com C vs. L	P_Val ue Com C vs. M	P_Val ue Com C vs. H
PARATHYROID GLA	Adenoma	1	0	0	0	1.0000	1.0000	1.0000	1.0000
PITUITARY GLAND	Adenoma of pars distalis	27	10	10	6	0.9662	0.8368	0.7999	0.9857
	Adenoma of pars intermedia	1	0	2	0	0.5577	1.0000	0.2283	1.0000
PREPUTIAL GLAND	Adenocarcinoma	0	0	1	0	0.3874	.	0.3111	.
	Leiomyoma	0	1	0	0	0.5830	0.3212	.	.
SALIV. GLANDS MA	Benign myoepithelioma	0	0	0	1	0.1982	.	.	0.3212
SALIV. GLANDS PA	Acinar cell Adenoma	1	0	0	0	1.0000	1.0000	1.0000	1.0000
SKIN/SUBCUTIS	Basal cell carcinoma	0	1	0	1	0.1910	0.3162	.	0.3212
	Benign fibrous histiocytoma	0	0	1	0	0.3874	.	0.3111	.
	Fibroma	3	0	0	1	0.5968	1.0000	1.0000	0.7981
	Hemangiosarcoma	0	0	2	1	0.1220	.	0.0952	0.3212
	Keratoacanthoma	10	5	3	6	0.3092	0.5626	0.8341	0.4267
	Keratoacanthoma+squamous_cell_car	10	6	6	7	0.2472	0.4088	0.3726	0.2930
	Lipoma	1	1	2	1	0.3259	0.5340	0.2283	0.5474
	Sarcoma not otherwise specified	2	1	0	1	0.5315	0.6835	1.0000	0.6905
SQUAMOUS CELL CARCINOMA	Squamous cell carcinoma	0	1	3	1	0.2698	0.3212	0.0286*	0.3261
	Trichoepithelioma	1	0	2	0	0.5577	1.0000	0.2283	1.0000
SPLEEN	Hemangioma	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	Sarcoma (not otherwise specified)	0	1	0	0	0.5811	0.3162	.	.
SYSTEMIC NEOPLA	Malignant lymphoma, pleomorphic	3	3	3	1	0.6817	0.2884	0.2787	0.7952
TESTES	Benign Leydig cell tumor	2	3	2	1	0.5853	0.1812	0.3675	0.6905
THYMUS	Benign thymoma	4	0	4	3	0.1724	1.0000	0.2087	0.4007
	Malignant thymoma	0	0	1	0	0.3874	.	0.3111	.
THYROID GLAND	C-cell adenoma	4	0	0	3	0.1482	1.0000	1.0000	0.4123
	Follicular cell adenoma	3	3	3	4	0.1182	0.2929	0.2733	0.1493
	Follicular cell carcinoma	4	1	2	0	0.8914	0.8556	0.6079	1.0000
	Follicular_cell_adnoma+carcinoma	7	4	5	4	0.4068	0.4947	0.2999	0.4947
ZYMBAL'S GLANDS	Sebaceous carcinoma, with abscess	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	Squamous cell carcinoma	2	0	0	0	1.0000	1.0000	1.0000	1.0000

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Com C: Combined Control

**Table 3B: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons  
Female Rats**

Organ Name	Tumor Name	0mg Com C N=102	10mg Low N=51	25mg Med N=51	50mg High N=51	P_Val ue Dose Resp	P_Val ue Com C vs. L	P_Val ue Com C vs. M	P_Val ue Com C vs. H
ADRENAL CORTICE	Adenoma	2	0	1	0	0.7353	1.0000	0.6764	1.0000
ADRENAL MEDULLA	Benign medullary tumor, complex p	0	1	0	0	0.5613	0.3261	.	.
BRAIN	Granular cell tumor	1	0	0	1	0.3182	1.0000	1.0000	0.4480
EYES	Rhabdomyosarcoma, of periocular t	1	0	0	0	1.0000	1.0000	1.0000	1.0000
HARDERIAN GLAND	Adenocarcinoma, anaplastic	0	1	0	0	0.5613	0.3261	.	.
JEJUNUM W/PEYER	Leiomyoma	1	0	0	2	0.0778	1.0000	1.0000	0.1608
LIVER	Cholangioma	1	0	0	1	0.3182	1.0000	1.0000	0.4480
	Hepato_adnoma+carcinoma	2	0	0	0	1.0000	1.0000	1.0000	1.0000
	Hepatocellular adenoma	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	Hepatocellular carcinoma	2	0	0	0	1.0000	1.0000	1.0000	1.0000
LYMPH NODE MESE	Hemangioma	1	0	0	1	0.3182	1.0000	1.0000	0.4480
LYMPH NODES	Hemangiosarcoma	1	0	0	0	1.0000	1.0000	1.0000	1.0000
MAMMARY GLAND A	Adenocarcinoma	11	6	2	0	0.9943	0.4907	0.9523	1.0000
	Fibroadenoma, fibromatous	40	18	3	4	1.0000	0.7143	1.0000	0.9998
	Lipoma, with a cyst	1	1	0	0	0.8087	0.5474	1.0000	1.0000
NASAL CAVITY	Squamous cell carcinoma, slide 26	0	1	0	0	0.5613	0.3261	.	.
OVARIES	Benign granulosa cell tumor	0	0	2	0	0.3182	.	0.0952	.
	Malignant granulosa cell tumor	0	0	1	0	0.3521	.	0.3162	.
PANCREAS	Islet cell adenoma	2	1	0	0	0.9171	0.6972	1.0000	1.0000
	Islet cell carcinoma	1	1	2	0	0.5074	0.5474	0.2283	1.0000
	Islet_cell_adnoma+carcinoma	3	2	2	0	0.7757	0.5269	0.4964	1.0000
PITUITARY GLAND	Adenoma of pars distalis	51	26	15	6	0.9999	0.4007	0.9832	0.9998
	Carcinoma of pars distalis	1	0	0	0	1.0000	1.0000	1.0000	1.0000
RECTUM	Stromal polyp	1	0	0	0	1.0000	1.0000	1.0000	1.0000
SALIV. GLANDS SU	Adenoma	0	0	0	1	0.1509	.	.	0.2560
SKIN/SUBCUTIS	Basal cell carcinoma	1	0	1	0	0.5752	1.0000	0.5239	1.0000
	Fibroma	1	1	0	0	0.8087	0.5474	1.0000	1.0000
	Fibrosarcoma	0	0	1	0	0.3521	.	0.3162	.
	Hemangioma	0	0	1	0	0.3491	.	0.3111	.
	Hemangiosarcoma, associated with	1	1	0	0	0.8087	0.5474	1.0000	1.0000
	Keratoacanthoma	1	0	1	0	0.5773	1.0000	0.5270	1.0000

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Com C: Combined Control



**Table 4A: Intercurrent Mortality Rate in Male Mice**

Week	Veh C#		5 mg		30 mg		100 mg		400 mg	
	No. of Death	Cum. %								
0 - 52	1	1.67	2	3.33	.	.	1	1.67	1	1.67
53 - 78	.	.	1	5.00	.	.	1	3.33	.	.
79 - 91	1	3.33	1	6.67	.	.	2	6.67	4	8.33
92 - 104	2	6.67	3	11.67	3	5.00	4	13.33	3	13.33
Ter. Sac.	56	93.33	53	88.33	57	95.00	52	86.67	52	86.67
Total	N=60									

# Veh C: Vehicle Control

**Table 4B: Intercurrent Mortality Rate Female Mice**

Week	Veh C#		5 mg		30 mg		100 mg		400 mg	
	No. of Death	Cum. %								
0 - 52	2	3.33	.	.	3	5.00	.	.	4	6.67
53 - 78	3	8.33	5	8.33	1	6.67	5	8.33	29	55.00
79 - 91	3	13.33	4	15.00	2	10.00	9	23.33	.	.
92 - 104	9	28.33	10	31.67	8	23.33	4	30.00	.	.
Ter. Sac.	43	71.67	41	68.33	46	76.67	42	70.00	27##	45.00
Total	N=60									

# Veh Cont: Vehicle Control, ## Female mice high dose group was terminated at Week 79

**Table 5A: Intercurrent Mortality Comparison Male Mice**

Test	Statistic	P_Value#
Dose-Response	Likelihood Ratio	0.2826
Homogeneity	Log-Rank	0.3550

#P-Values were calculated using data from Vehicle Control, Low, Medium, Mid-Hi, and High dose groups

**Table 5B: Intercurrent Mortality Comparison Female Mice**

Test	Statistic	P_Value#
Dose-Response	Likelihood Ratio	<.0001
Homogeneity	Log-Rank	<.0001

#P-Values were calculated using data from Vehicle Control, Low, Medium, Mid-Hi, and High dose groups

**Table 6A: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons Male Mice**

Organ Name	Tumor Name	0mg	5mg	30mg	100mg	400mg	P_Val ue	P_Val ue VC vs L	P_Val ue VC vs M	P_Val ue VCvs MH	P_Val ue VCvs H
		Veh C N=60	Low N=60	Med N=60	Mi d-Hi N=60	Hi gh N=60	Dose Resp				
ADRENAL CORTICE	A-cell adenoma	1	1	0	0	1	0.4625	0.7434	0.5043	0.4957	0.7478
	B-cell adenoma	5	0	0	0	4	0.0866	0.9688	0.9727	0.9701	0.4889
	Carcinoma	1	0	0	0	1	0.3583	0.4912	0.5043	0.4957	0.7478
ADRENAL MEDULLA	Pheochromocytoma	0	1	0	0	0	0.6028	0.4912	.	.	.
BODY CAVITIES	Hemangioma	0	0	1	0	1	0.2000	.	0.5043	.	0.4957
	Metastatic sarcoma	0	0	0	1	0	0.1986	.	.	0.4957	.
BONE	Osteosarcoma	0	0	0	0	1	0.1986	.	.	.	0.4957
CECUM	Adenoma	0	0	0	0	1	0.1986	.	.	.	0.4957
	Leiomyoma	0	1	0	0	0	0.6028	0.4912	.	.	.
COLON	Adenoma	0	0	1	0	0	0.3972	.	0.5043	.	.
DUODENUM	Adenocarcinoma	0	0	1	0	0	0.3972	.	0.5043	.	.
	Adenocarcinoma+adenoma	2	1	2	0	2	0.3898	0.4867	0.3157	0.7478	0.6842
	Adenoma	2	1	1	0	2	0.3231	0.4867	0.5065	0.7478	0.6842
DUODENUM+ILEUM+ JEJUNUM	Adenoma	3	1	1	0	2	0.4164	0.6775	0.6972	0.8750	0.4917
	Adnoma+carcinoma	4	1	2	1	2	0.5734	0.8069	0.6685	0.8126	0.6521
	Carcinoma	1	0	1	1	0	0.6383	0.4912	0.2521	0.7478	0.4957
EPIDIDYMIDES	Interstitial cell tumor	1	0	0	0	1	0.3583	0.4912	0.5043	0.4957	0.7478
	Schwannoma	0	0	0	1	0	0.1986	.	.	0.4957	.
HARDERIAN GLAND	Adenocarcinoma	5	0	0	1	2	0.4571	0.9688	0.9727	0.8928	0.7736
	Adenoma	2	1	1	2	2	0.3144	0.4867	0.5065	0.6842	0.6842
ILEUM	Carcinoma	1	0	1	0	0	0.7597	0.4912	0.2521	0.4957	0.4957
JEJUNUM	Adenoma	1	0	0	0	0	0.7979	0.4912	0.5043	0.4957	0.4957
	Adnoma+carcinoma	1	0	0	1	0	0.5569	0.4912	0.5043	0.7478	0.4957
	Carcinoma	0	0	0	1	0	0.1986	.	.	0.4957	.
KIDNEYS	Tubular adenoma	0	1	0	1	0	0.4792	0.4912	.	0.4957	.
	Tubular carcinoma	1	0	0	0	0	0.7979	0.4912	0.5043	0.4957	0.4957
LIVER	All hepatocell. tum.	11	15	14	17	19	0.0621	0.2362	0.3617	0.1389	0.0612
	Hemangiosarcoma	0	1	2	0	0	0.7908	0.4912	0.2521	.	.
	Hepatocellular adenoma, multiple	9	14	9	14	14	0.1739	0.1638	0.4147	0.1759	0.1638
	Hepatocellular carcinoma, hepatob	6	5	8	4	7	0.3520	0.4751	0.4149	0.6176	0.4865
	Hepatocellular adenoma+carcinoma	11	15	14	17	19	0.0621	0.2362	0.3617	0.1389	0.0612
LUNG	Alveolar/bronchiolar adenoma	7	6	7	7	3	0.9148	0.4728	0.4017	0.5982	0.8321

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Veh C: Vehicle Control

**Table 6A: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons  
Male Mice**

Organ Name	Tumor Name	0mg	5mg	30mg	100mg	400mg	P_Val ue	P_Val ue VC vs L	P_Val ue VC vs M	P_Val ue VCvs MH	P_Val ue VCvs H
		Veh C N=60	Low N=60	Med N=60	Mi d-Hi N=60	Hi gh N=60	Dose Resp				
LUNG	Alveolar/bronchiolar carcinoma	10	4	6	5	2	0.9737	0.9188	0.8004	0.8581	0.9842
MAMMARY GLAND A	Hemangioma, subcutaneous	0	1	0	0	1	0.2778	0.4912	.	.	0.4957
MESENT. LYMPH N	Fibrous histiocytic sarcoma	0	0	1	0	0	0.3958	.	0.5085	.	.
	Hemangioma	0	0	1	0	1	0.2000	.	0.5043	.	0.4957
	Hemangiosarcoma	0	0	0	2	0	0.3583	.	.	0.2435	.
PITUITARY GLAND	Adenoma, pars anterior	0	1	1	0	0	0.6792	0.4912	0.5043	.	.
PREPUTIAL GLAND	Squamous carcinoma	1	0	0	0	0	0.7979	0.4912	0.5043	0.4957	0.4957
SKIN/SUBCUTIS	Fibroma	1	0	0	0	0	0.7979	0.4912	0.5043	0.4957	0.4957
	Hemangiosarcoma	0	0	0	1	0	0.1986	.	.	0.4957	.
	Keratoacanthoma+Squamous_carcinoma	0	0	2	0	0	0.6375	.	0.2521	.	.
	Keratoacanthoma	0	0	1	0	0	0.3972	.	0.5043	.	.
	Schwannoma, auricular (likely amelanotic)	0	0	0	1	1	0.1181	.	.	0.4957	0.4957
SPLEEN	Squamous carcinoma	0	0	1	0	0	0.3972	.	0.5043	.	.
	Hemangioma	1	0	0	0	0	0.7979	0.4912	0.5043	0.4957	0.4957
SPLEEN	Hemangiosarcoma	0	2	3	1	0	0.8629	0.2391	0.1250	0.4957	.
	SYSTEMIC NEOPLASIA	Histiocytic sarcoma	2	1	0	2	0	0.7786	0.4934	0.7564	0.6842
SYSTEMIC NEOPLASIA	Malignant lymphoma	3	5	3	1	1	0.9379	0.3387	0.3559	0.6842	0.6842
	Mastocytoma	0	0	1	0	0	0.3972	.	0.5043	.	.
	Myeloid leukemia	0	0	1	0	0	0.3972	.	0.5043	.	.
TESTES	Leydig cell tumor	1	0	0	0	0	0.7979	0.4912	0.5043	0.4957	0.4957
THYROID GLAND	Follicular carcinoma	1	0	0	0	1	0.3583	0.4912	0.5043	0.4957	0.7478

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Veh C: Vehicle Control



**Table 6B: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons  
Female Mice Excluding the High Dose Group**

Organ Name	Tumor Name	0mg Veh C N=60	5mg Low N=60	30mg Med N=60	100mg High N=60	P_Val ue Dose Resp	P_Val ue Veh C vs L	P_Val ue Veh C vs M	P_Val ue Veh Cvs MH
SKIN/SUBCUTIS	Fibrosarcoma	0	1	0	0	0.7500	0.5000	.	.
	Hemangioma	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	Squamous carcinoma	0	0	1	0	0.5000	.	0.5047	.
SPIINAL CORD, TH	Fibroma	0	0	0	1	0.2453	.	.	0.4952
SPLEEN	Hemangioma	0	0	1	0	0.5000	.	0.5047	.
	Hemangiosarcoma	0	0	1	1	0.1848	.	0.5047	0.4952
STOMACH	Papilloma, forestomach	0	0	0	2	0.0593	.	.	0.2429
SYSTEMIC NEOPLA	Histiocytic sarcoma	1	3	4	2	0.4789	0.3089	0.1874	0.4857
	Malignant lymphoma	23	24	22	20	0.6965	0.5639	0.6508	0.7354
	Myeloid leukemia	0	0	1	0	0.5000	.	0.5047	.
THYMUS	Thymoma, lymphocytic	0	0	1	0	0.5000	.	0.5047	.
THYROID GLAND	Follicular adenoma	0	0	0	1	0.2453	.	.	0.4952
	Follicular carcinoma	1	0	0	0	1.0000	1.0000	1.0000	1.0000
	Follicular adenoma+carcinoma	1	0	0	1	0.4295	1.0000	1.0000	0.7429
TONGUE	Papilloma, inverted	0	0	0	1	0.2453	.	.	0.4952
UTERUS	Adenocarcinoma	1	0	2	0	0.6848	1.0000	0.5071	1.0000
	Adenocarcinoma+adenoma	1	0	3	0	0.7146	1.0000	0.3160	1.0000
	Adenoma	0	0	1	0	0.5000	.	0.5047	.
	Endometrial polyp	3	3	2	4	0.2874	0.6518	0.8184	0.4788
	Glandular polyp	1	3	2	0	0.8970	0.3089	0.5071	1.0000
	Hemangioma	0	1	1	0	0.6232	0.5000	0.5047	.
	Hemangiosarcoma	0	2	0	1	0.4313	0.2523	.	0.4952
	Leiomyoma	0	1	0	2	0.1047	0.5000	.	0.2429
	Leiomyosarcoma	2	0	2	1	0.5238	1.0000	0.6981	0.8750
	Malignant schwannoma	0	0	0	1	0.2453	.	.	0.4952
	Stromal sarcoma	0	0	1	1	0.1848	.	0.5047	0.4952
	Yolk sac carcinoma	1	0	0	0	1.0000	1.0000	1.0000	1.0000

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Veh C: Vehicle Control

**Table 6C: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons  
Female Mice Including the High dose Group**

Organ Name	Tumor Name	0mg	5mg	30mg	100mg	400mg	P_Val ue	P_Val ue VC vs L	P_Val ue VC vs M	P_Val ue VCvs MH	P_Val ue VCvs H
		Veh C N=60	Low N=60	Med N=60	Mi d-Hi N=60	Hi gh N=60	Dose Resp				
ADRENAL CORTICE	A-cell adenoma	1	0	1	1	0	0.4606	0.5000	0.2523	0.7476	0.2740
	Adenoma, cortical	0	0	0	1	0	0.3103	.	.	0.4952	.
	Carcinoma	0	1	0	0	0	0.5408	0.5047	.	.	.
	Hemangioma	0	0	0	1	0	0.3103	.	.	0.4952	.
ADRENAL MEDULLA	Pheochromocytoma	0	0	1	1	0	0.2148	.	0.5047	0.4952	.
BODY CAVITIES	Hemangioma	0	0	0	1	0	0.3103	.	.	0.4952	.
CECUM	Leiomyoma	1	0	0	0	0	0.7716	0.5000	0.5047	0.4952	0.2740
CEREBRUM	Lipoma	1	0	0	0	0	0.7716	0.5000	0.5047	0.4952	0.2740
CERVIX	Adenocarcinoma	0	0	0	1	0	0.3103	.	.	0.4952	.
	Leiomyoma	1	0	0	0	0	0.7716	0.5000	0.5047	0.4952	0.2740
DUODENUM	Adenoma	0	0	0	1	0	0.3103	.	.	0.4952	.
DUODENUM+ILEUM	Adenoma	0	0	0	1	1	0.0459	.	.	0.4952	0.2740
HARDERIAN GLAND	Adenocarcinoma	1	1	0	3	0	0.4092	0.7524	0.5047	0.3017	0.2740
	Adenoma	3	1	1	4	0	0.6134	0.6911	0.6911	0.4788	0.6174
ILEUM	Adenoma	0	0	0	0	1	0.0862	.	.	.	0.2740
	Hemangiosarcoma	0	0	0	1	0	0.3103	.	.	0.4952	.
LIVER	All hepatocellular tum.	4	2	4	2	0	0.8939	0.6518	0.6421	0.6426	0.7251
	Hemangioma	1	0	1	0	0	0.6855	0.5000	0.2523	0.4952	0.2740
	Hemangiosarcoma	0	0	1	0	0	0.3090	.	0.5093	.	.
	Hepato_adnoma+carcinoma	4	2	5	2	0	0.9044	0.6518	0.5000	0.6426	0.7251
	Hepatocellular adenoma	1	1	4	1	0	0.7464	0.7524	0.1874	0.7476	0.2740
	Hepatocellular carcinoma	3	1	3	1	0	0.8647	0.6840	0.6608	0.6767	0.6174
LUNG	Alveolar/bronchial adenoma	1	1	0	1	0	0.5679	0.7524	0.5047	0.7476	0.2740
	Alveolar/bronchial carcinoma	2	1	2	2	0	0.6521	0.5000	0.6911	0.6767	0.4702
MAMMARY GLAND A	Adenocarcinoma	2	1	1	2	0	0.6162	0.4929	0.5000	0.6767	0.4702
	Adenoma	0	0	0	1	0	0.3103	.	.	0.4952	.
	Adenocarcinoma+adenoma	2	1	1	3	0	0.5581	0.4929	0.5000	0.4820	0.4702
MESENT. LYMPH N	Hemangioma	0	0	1	0	0	0.3103	.	0.5047	.	.
OVARIES	Cystadenocarcinoma	1	0	0	0	0	0.7716	0.5000	0.5047	0.4952	0.2740
	Cystadenocarcinoma+cystadenoma	2	2	1	2	0	0.7054	0.6911	0.5071	0.6839	0.4756
	Cystadenoma	1	2	1	2	0	0.5938	0.5000	0.2523	0.4928	0.2740

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Veh C: Vehicle Control

**Table 6C: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons Female Mice Including the High dose Group**

Organ Name	Tumor Name	0mg	5mg	30mg	100mg	400mg	P_Val ue	P_Val ue VC vs L	P_Val ue VC vs M	P_Val ue VCvs MH	P_Val ue VCvs H
		Veh C N=60	Low N=60	Med N=60	Mid-Hi N=60	High N=60	Dose Resp				
PANCREAS	I slet cell carci noma, block 2a	0	0	0	1	0	0.3103	.	.	0.4952	.
PITUITARY GLAND	Adenoma, pars anterior	7	6	9	10	0	0.9177	0.5000	0.4252	0.2838	0.9054
	Adenoma: pars intermedia	0	0	0	1	0	0.3103	.	.	0.4952	.
SKIN/SUBCUTIS	Fibrosarcoma	0	1	0	0	0	0.5431	0.5000	.	.	.
	Hemangi oma	1	0	0	0	0	0.7716	0.5000	0.5047	0.4952	0.2740
	Squamous carci noma	0	0	1	0	0	0.3103	.	0.5047	.	.
SPI NAL CORD, TH	Fi broma	0	0	0	1	0	0.3103	.	.	0.4952	.
SPLEEN	Hemangi oma	0	0	1	0	0	0.3103	.	0.5047	.	.
	Hemangi osarcoma	0	0	1	1	1	0.0604	.	0.5047	0.4952	0.2740
STOMACH	Papi l loma, forestomach	0	0	0	2	0	0.2148	.	.	0.2429	.
SYSTEMI C NEOPLA	Hi stiocyti c sarcoma	1	3	4	2	0	0.7828	0.3089	0.1874	0.4857	0.2703
	Mal i gnan t l ymphoma	23	24	22	20	0	0.9999	0.5639	0.5000	0.5934	0.9998
	Myeloi d l eukemi a	0	0	1	0	0	0.3103	.	0.5047	.	.
THYMUS	Thymoma, l ymphocyt i c	0	0	1	0	0	0.3103	.	0.5047	.	.
THYROI D GLAND	Fol l i cul ar adenoma	0	0	0	1	0	0.3103	.	.	0.4952	.
	Fol l i cul ar carci noma	1	0	0	0	0	0.7682	0.4953	0.5000	0.4906	0.2703
	Fol l i cul ar_adnoma+carci noma	1	0	0	1	0	0.4196	0.4953	0.5000	0.7429	0.2703
TONGUE	Papi l loma, i nverted	0	0	0	1	0	0.3103	.	.	0.4952	.
UTERUS	Adenocarci noma	1	0	2	0	0	0.7229	0.5000	0.5071	0.4952	0.2740
	Adenoma	0	0	1	0	0	0.3103	.	0.5047	.	.
	Adenocarci noma+adnoma	1	0	3	0	0	0.7903	0.5000	0.3160	0.4952	0.2740
	Endometri al pol yp	3	3	2	4	1	0.4205	0.6518	0.5000	0.4788	0.2940
	Gi andul ar pol yp	1	3	2	0	0	0.9317	0.3089	0.5071	0.4952	0.2740
	Hemangi oma	0	1	1	0	0	0.5787	0.5000	0.5047	.	.
	Hemangi osarcoma	0	2	0	1	1	0.1524	0.2523	.	0.4952	0.2740
	Lei omyoma	0	1	0	2	0	0.3177	0.5000	.	0.2429	.
	Lei omyosarcoma	2	0	2	1	0	0.6787	0.7524	0.3160	0.4928	0.4756
	Lei omyosarcoma+stromal _sarcoma	2	0	3	2	0	0.6230	0.7524	0.5089	0.6839	0.4756
	Mal i gnan t schwannoma	0	0	0	1	0	0.3103	.	.	0.4952	.
	Stromal sarcoma	0	0	1	1	0	0.2148	.	0.5047	0.4952	.
	Yol k sac carci noma	1	0	0	0	0	0.7682	0.4953	0.5000	0.4906	0.2703

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Veh C: Vehi cle Control

Figure 1A: Kaplan-Meier Survival Functions for Male Rats

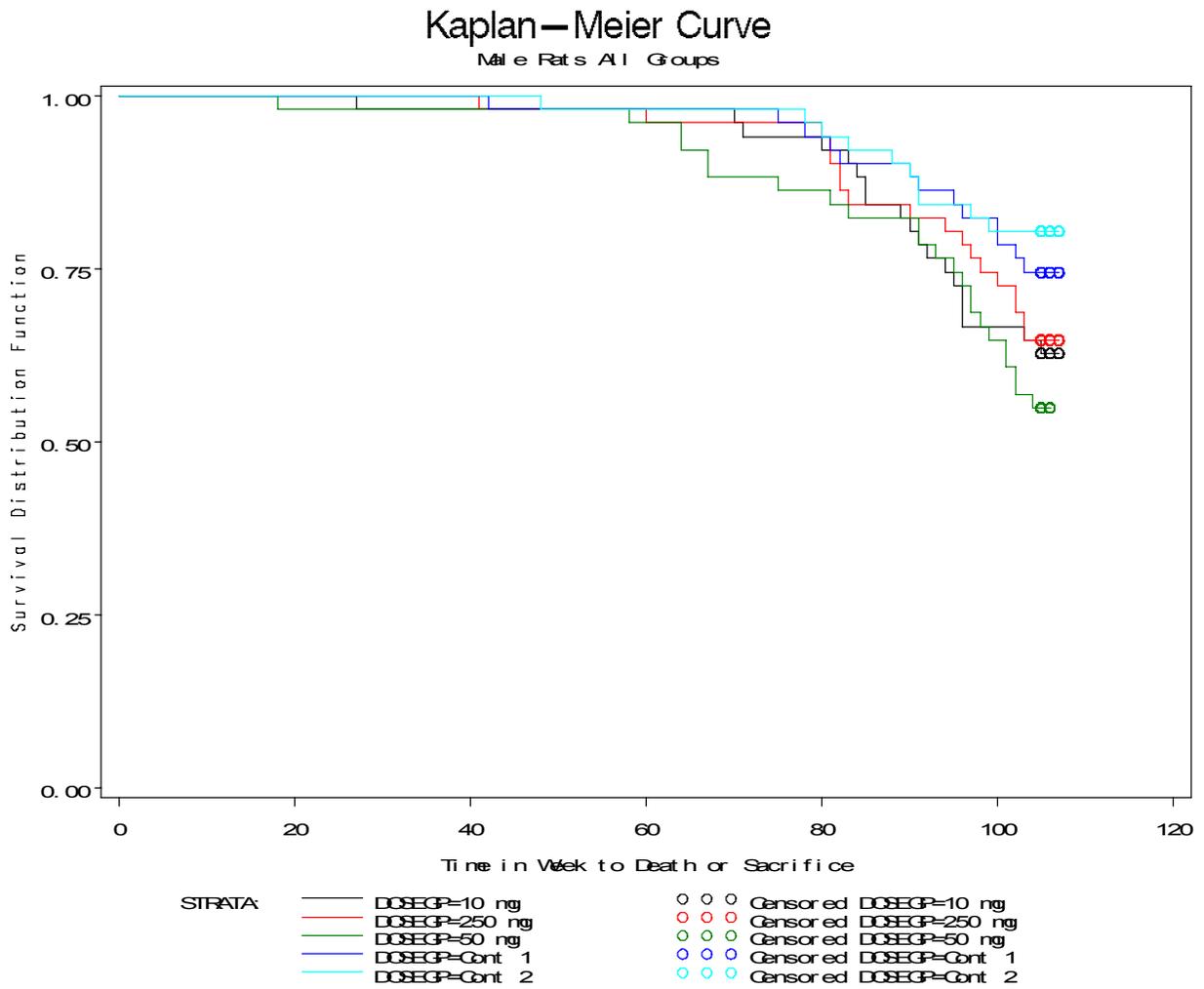


Figure 1B: Kaplan-Meier Survival Functions for Female Rats

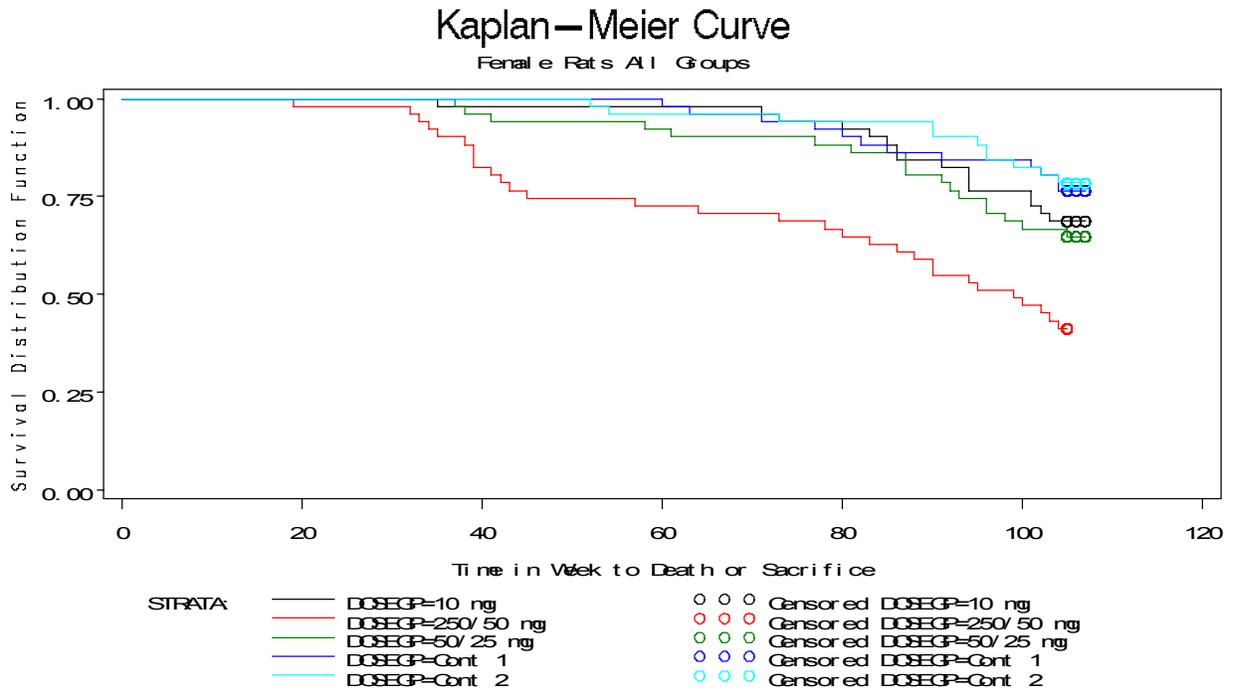


Figure 2A: Kaplan-Meier Survival Functions for Male Mice

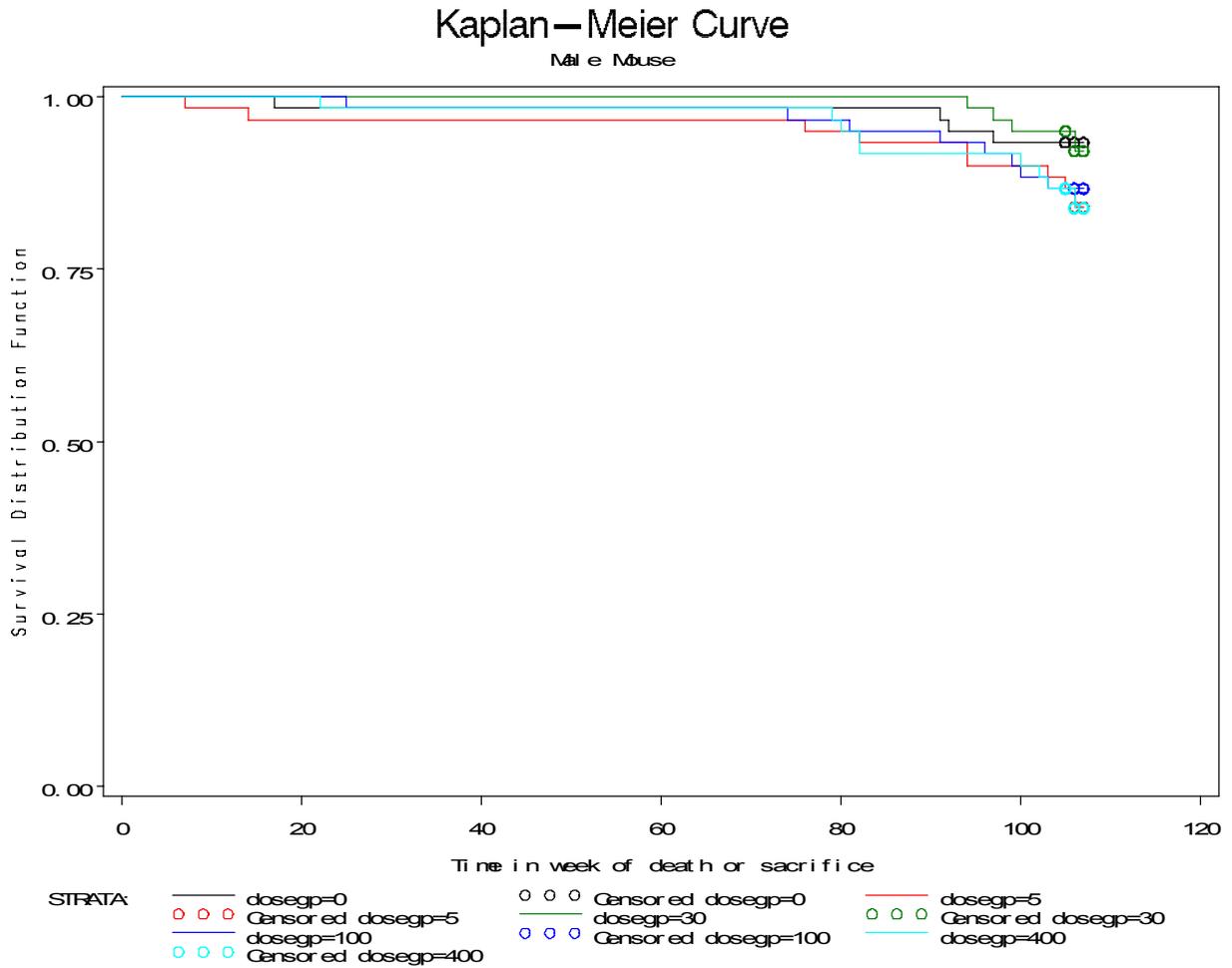
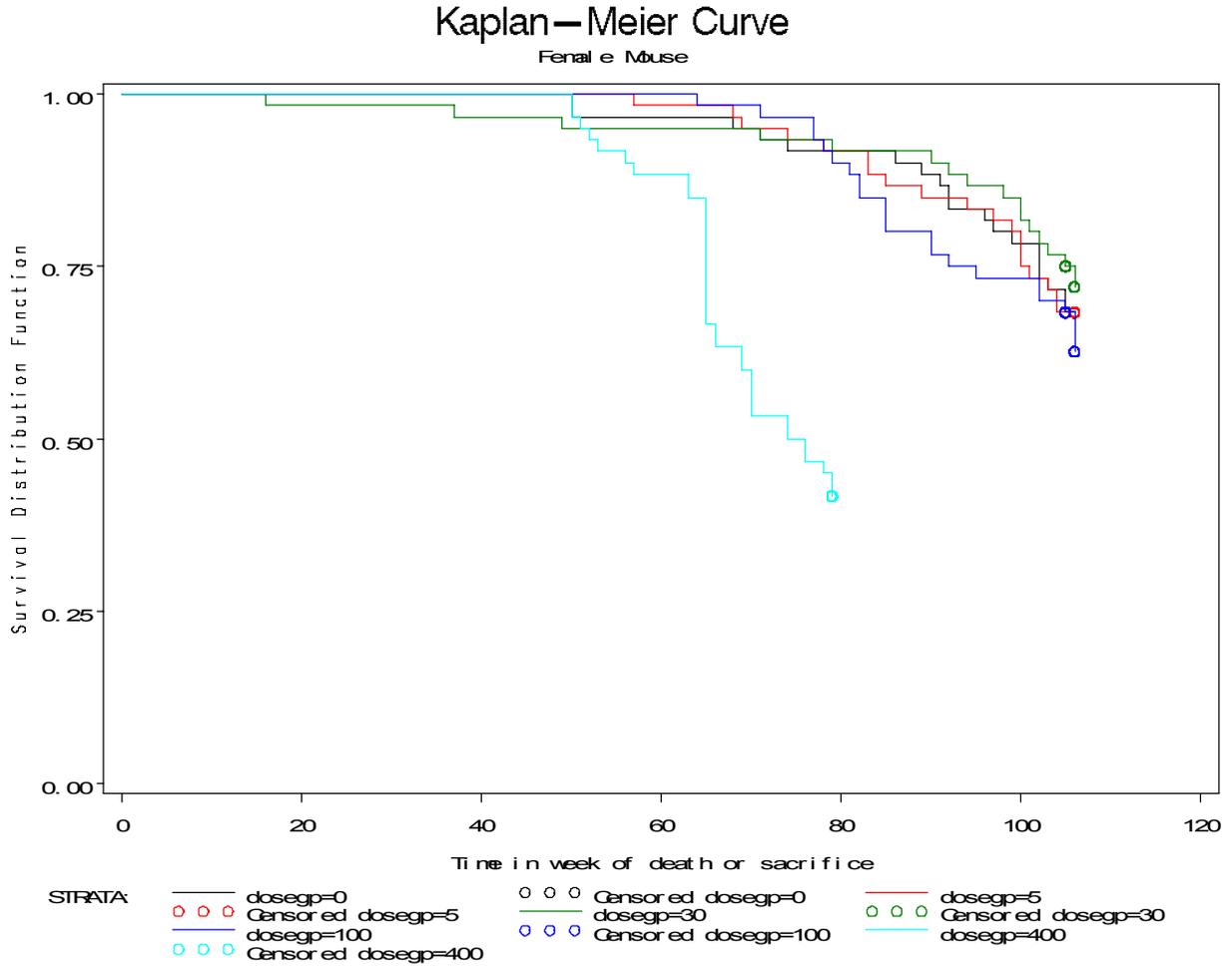


Figure 2B: Kaplan-Meier Survival Functions for Female Mice



## 7. References

1. Peto, R., M.C. Pike, N.E. Day, R.G. Gray, P.N. Lee, S. Parish, J. Peto, Richards, and J. Wahrendorf, "Guidelines for sample sensitive significance test for carcinogenic effects in long-term animal experiments", Long term and short term screening assays for carcinogens: A critical appraisal, International agency for research against cancer monographs, *Annex to supplement, World Health Organization, Geneva*, 311-426, 1980.
2. Bailer AJ, Portier CJ (1988). "Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples." *Biometrics*, 44, 417-431.
3. Bieler, G. S. and Williams, R. L. (1993). "Ratio estimates, the delta method, and quantal response tests for increased carcinogenicity". *Biometrics* 49, 793-801.
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/s/  
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MOHAMMAD A RAHMAN  
03/15/2013

KARL K LIN  
03/15/2013  
Concur with review

## STATISTICS FILING CHECKLIST FOR A NEW NDA/BLA

**NDA Number: 204-410**

**Applicant: Actelion**

**Stamp Date: 10/19/2012**

**Drug Name: Macitentan**

**NDA/BLA Type: standard**

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

	<b>Content Parameter</b>	<b>Yes</b>	<b>No</b>	<b>NA</b>	<b>Comments</b>
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.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

<b>Content Parameter (possible review concerns for 74-day letter)</b>	<b>Yes</b>	<b>No</b>	<b>NA</b>	<b>Comment</b>
Designs utilized are appropriate for the indications requested.	<b>x</b>			The sponsor censored patients at the end of treatment instead of at the end of study. This will be a review issue.
Endpoints and methods of analysis are specified in the protocols/statistical analysis plans.	<b>x</b>			
Interim analyses (if present) were pre-specified in the protocol and appropriate adjustments in significance level made. DSMB meeting minutes and data are available.	<b>x</b>			No interim analysis on efficacy was performed.
Appropriate references for novel statistical methodology (if present) are included.			<b>x</b>	
Safety data organized to permit analyses across clinical	<b>x</b>			

File name: 5\_Statistics Filing Checklist for a New NDA\_BLA110207

## STATISTICS FILING CHECKLIST FOR A NEW NDA/BLA

trials in the NDA/BLA.				
Investigation of effect of dropouts on statistical analyses as described by applicant appears adequate.	<b>x</b>			

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Reviewing Statistician

Date

---

Supervisor/Team Leader

Date

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/s/  
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JIALU ZHANG  
11/19/2012

HSIEN MING J HUNG  
11/28/2012