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APPLICATION NUMBER:

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STATISTICAL REVIEW(S)

JOINT CLINICAL/STATISTICAL REVIEW
(ADDENDUM)

NDA #:	203,496
Applicant:	United Therapeutics
Name of Drug:	Treprostinil tablets
Indication:	Pulmonary arterial hypertension
Date of submission:	October 23, 2011
Statistical Reviewer:	John Lawrence, Ph.D. (HFD–710)
Medical Reviewer:	Maryann Gordon, M.D. (HFD–110)

The purpose of this addendum is to provide some additional analyses of the pivotal Phase 3 efficacy study TDE-PH-302. As noted on p. 24 of the original review, at the end of study (Week 12), the estimated placebo-subtracted change in 6-minute walking distance was 25.5 m (p=0.0001) using the sponsor's single imputation method and their adjudication of reasons for dropout. However, 59 subjects (25%) in the UT-15C group did not have the week 12 walk test compared to 18 subjects (11%) in the placebo group. When the 59 UT-15C subjects are given worst rank, the p-value becomes 0.92. When the missing placebo subjects are assigned worst rank as well, the p-value becomes 0.21. It is of great concern that UT-15C had more than double dropout rate than placebo.

14 (6%) subjects in the treprostinil group and 9 (8%) subjects in the placebo group died during the course of the study before the walking distance could be measured at Week 12 (10 and 6 deaths respectively were listed as the reason for discontinuation but others died after discontinuation during 12 week period). Because of the 2:1 randomization, the percentage of deaths was approximately balanced between the two groups. There were an additional 45 (19%) subjects with missing data at week 12 in the treprostinil group and an additional 9 (8%) in the placebo group. Only 3 subjects total had missing data for the reason "In study, too ill to walk". 100% of the subjects that did not die and were not too ill to walk should have had a week 12 followup visit where the walking distance should have been measured and this value should have been used in the ITT analysis regardless of whether the subject took their randomized treatment.

The primary analysis was complicated, but essentially all subjects were assigned a score between 0 and 1 based on their change from baseline walking distance. Higher scores indicate better change in walking distance. The average imputed score for the 59 treprostinil subjects with missing Week 12 data is 0.36 while the average score for the 18 placebo subjects is 0.11. From this, it is seen that there was a large amount of missing data and the way it was handled seemed to substantially favor showing a treatment effect (i.e., 0.36 vs. 0.11). Since it is preferable to make decisions based on observed data rather than made up data and there was a substantial amount of missing data in this study with twice as much missing in the treatment group, it seems worthwhile to consider other ways of handling the missing data that do not favor showing a treatment effect.

In this addendum, three additional analyses are considered:

1. Analysis of change from baseline to Week 4 (earlier time point than used in the primary analysis) using other imputation methods; giving worst rank to all subjects (treprostinil and placebo) with missing data or only to all subjects in the trepostinil group.

In the sponsor's ITT analysis of change from baseline to Week 4, the estimated placebo subtracted change from baseline is 14 m with a p-value of 0.0025. 25 subjects in the treprostinil group and 8 in the placebo group had missing value sat Week 4. In the sponsor's analysis, the imputed scores for these 25 subjects with missing values had a mean of 0.34 while the mean score for the 8 placebo subjects was 0.08. Again, the sponsor’s imputation seemed to substantially favor the treprostinil group.

When these 25 subjects in the treprostinil group are all given the worst score, the point estimate of the placebo subtracted change from baseline is 10 m and the p-value is 0.063. If, in addition, the 8 placebo subjects are also given the worst score, then the p-value remains 0.063 (note: 7 of them already had the worst rank, so it only changes the rank for 1 placebo subject).

2. Analysis of change from baseline to Week 12 giving fewer subjects from treprostinil group the worst rank (i.e. not all 59 are given worst rank).

When all 59 subjects in the treprostinil group are given the worst score, the p-value is 0.92. If only the top 23 of these 59 are given the worst score and the remaining 36 scores are left "as is", the average score for all 59 treprostinil subjects with missing data is 0.13 compared to an average score of 0.11 for the placebo subjects with missing data. The p-value for the analysis with this imputation is 0.051. 23 is the smallest number of subjects in the treprostinil group with missing data who were not already given the worst rank that would have to be given the worst rank to make the p-value above 0.05.

The reasons given for missing data at Week 12 for these 23 subjects were: Adverse event (14), Consent withdrawn (2), Discontinued for other reasons (1), In study, unblinded or other (3), Lost to follow-up (3).

3. Multiple imputation method for missing values at Week 12.

Another approach is to use multiple imputation to impute the missing data. For each imputed dataset, the worst rank was given to subjects who died as was done in the original single imputation analysis. For subjects with missing value who did not die, a random score was chosen uniformly between 0 and 0.25; this is based on the concept that anyone with missing data would have fallen in the lowest quartile had they been coerced into actually doing the walk test at Week 12. Note that there are 45 subjects in the treatment group who have missing data at Week 12 for reason other than death and 9 in the placebo group. The average score in the sponsor's analysis for the 45 subjects was 0.45 and the average score for the 9 placebo subjects was 0.16. From this imputed

dataset, the stratified treatment effect was estimated and then combined using a multiple imputation formula (Rubin, D.B. (1987) *Multiple Imputation for Nonresponse in Surveys*. J. Wiley & Sons, New York.). The multiple imputation analysis will have two important differences from the original single imputation. First, the imputed scores will have the same average value in both groups (namely, 0.125). Second, the uncertainty in imputing a value will be reflected in the variance. This uncertainty is not factored in when a single imputation is used and treated like a fixed value. The result of the multiple imputation analysis done this way is a p-value of 0.056. However, the validity of multiple imputation analysis relies on model assumptions including the assumption that the values are missing at random. This assumption implies that the large imbalance in the rate of missing data between the two treatment groups does not matter in statistical analysis, which can be very problematic in my view.

There are other ways of imputing the missing values as part of the multiple imputation analysis. In general, those imputation schemes that tend to give better values to subjects with missing data will tend to favor showing a treatment effect because the number of subjects affected by these better imputed values is higher in the treprostinil group compared to the placebo group and vice versa. For example, if the missing data from the patients who did not die or too ill to walk are imputed by a random score smaller than 0.125 on average, then the treatment difference will likely not be statistically significant.

In summary, the robustness of the efficacy results depends heavily on how the missing data are treated in statistical analysis; the p-value can range from 0.0001 (from the sponsor's analysis) to 0.92 (from analysis giving all treatment subjects with missing data the worst score). So in my opinion, the efficacy of the treprostrinil tablets has not been convincingly demonstrated, based on this study.

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/s/

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Office of Biostatistics

Statistical Review and Evaluation

CARCINOGENICITY STUDY

IND/NDA Number:	NDA 203-496
Drug Name:	UT-15C (Treprostinil Diethanolamine)
Indication(s):	26 Week Carcinogenicity Study in Tg.rasH2 mice
Applicant:	Sponsor: United Therapeutics Corporation 55 T. W. Alexander Drive, P.O. Box 14186 Research Triangle Park, NC 27709
	(b) (4) [redacted]
Documents Reviewed:	Electronic report submission: Dated Dec. 27, 2011 Electronic data submission: Dated January 31, 2012
Review Priority:	Standard
Biometrics Division:	Division of Biometrics -6
Statistical Reviewer:	Mohammad Atiar Rahman, Ph.D.
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Keywords:	Carcinogenicity, Dose response

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1. Background

In this submission the sponsor included a report of an animal carcinogenicity study in mice. This study was intended to assess the carcinogenic potential of UT-15C (Treprostinil Diethanolamine) in Tg.rasH2 mice when administered orally via gavage at appropriate drug levels for about 26 weeks. All surviving animals were sacrificed at Week 27. Results of this review have been discussed with the reviewing pharmacologist Dr. Joseph.

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. Design

Two separate experiments were conducted, one in male and one in female mice. In each of these two experiments there were three treated groups, one vehicle control group (negative control), and one positive control group. A total of one hundred Tg.rasH2 mice of each sex were randomly assigned to treated and vehicle control groups in equal size of 25 animals per group. The positive control group had 15 mice in each sex. The dose levels for treated groups were 5.0, 10.0, and 20.0 mg/kg/day for male mice and 3.0, 7.5, and 15.0 mg/kg/day for female mice. In this review the three treated groups are referred to as the low, medium, and high dose groups, respectively. The animals in the positive control group received a total of 3 urethane intraperitoneal injections on Study Days 1, 3, and 5. The animals in the vehicle control group received vehicle (sterile water for injection).

All animals were observed twice daily for morbidity and mortality. They were also observed daily for clinical signs of toxicity. A detailed hands-on examination was performed on all animals once a week. Body weights for individual animals were measured once weekly beginning on first day of dosing through Week 13 and biweekly thereafter.

2.1. Sponsor's analyses

2.1.1. Survival analysis

The sponsor presented a summary table of the mortalities of animals by sex. In the original protocol the sponsor proposed to analyze the mortality data using the Generalized Wilcoxon test. However, the submitted sponsor's reports do not contain results of any formal statistical analysis of mortality data.

Sponsor's findings: The sponsor analysis showed one male death in medium dose group, one female death in medium dose group and one female death in high dose group. Besides, one female mouse from the low dose group and one female mouse from the medium dose group were sacrificed in moribund condition. The sponsor concluded that there was no significant differences in mortality among treatment groups in either sex.

2.1.2. Tumor data analysis

The sponsor presented a summary table of the tumor findings by sex, including the (b) (4) historical control ranges. The tumor data were analyzed using the method proposed by Peto et al. (1980), incorporating the context of observation. The positive control was compared to the vehicle control using the one-sided Fisher's exact test.

Adjustment for multiple testing: No adjustment for multiple testing was performed.

Sponsor's findings: The sponsor's analysis did not show statistically significant dose response relationship among the treatment groups or increased incidence in the treated groups in any of the observed tumor types. The sponsor concluded that the incidences of all observed lesions were low and were within the historical control ranges established at (b) (4) laboratories, and the treatment by the test article did not increase the incidence of any neoplastic lesions.

The sponsor's analysis further showed statistically significant increased ($p < 0.05$) incidences of pulmonary tumors and splenic hemangiosarcomas in the positive control male and female mice when compared to their respective vehicle control.

2.2. Reviewer's analyses

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

2.2.1. Survival analysis

The survival distributions of animals in all four treatment groups (vehicle control, low, medium, and high dose groups) were estimated by the Kaplan-Meier product limit method. 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 intercurrent mortality data of all treatment groups are given in Tables 1A and 1B in the appendix for male and female mice, respectively. The Kaplan-Meier curves for survival rates of all treatment groups are given in Figures 1A and 1B in the appendix for male and female mice, 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 mice, respectively.

Reviewer's findings: This reviewer's analysis showed 0, 0, 1, and 0 deaths of male mice in vehicle control, low, medium, and high groups, respectively before the scheduled sacrifice on Week 27. In positive control group 7 male mice had natural death before Week 16 and the remaining 8 male mice were sacrificed on Week 16 as the part of part of a planned interim sacrifice. Similarly there were 0, 1, 2, and 1 deaths of female mice in vehicle control, low, medium, and high groups, respectively before the scheduled sacrifice on Week 27. In positive control group 4 female mice had natural death before Week 16 and the remaining 11 female mice were sacrificed on Week 16 as the part of part of a planned interim sacrifice. This reviewer's analysis did not show statistically significant dose response relationship in mortality across vehicle control, low, medium, and high dose groups in either sex. The pairwise comparisons also did not show statistically significant increased mortality in the low, medium, and high dose groups compared to the vehicle control group in either sex.

Reviewer's comment: *The sponsor's count showed a total of 3 deaths (1 male and 2 females), while this reviewer's count showed a total of 5 deaths (1 male and 4 females). These discrepancies are due to the fact that there were two female mice, one in low dose group and one in medium dose group, that were killed by the sponsor in their moribund conditions. In the submitted data sets these animals were coded as naturally dead, which is reflected in this reviewer's count.*

2.2.2. Tumor data analysis

The tumor data were analyzed for dose response relationships and pairwise comparisons of vehicle 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 $N^* = \Sigma s_h$. 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 N^* 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 being tested, 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 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 mice, respectively.

Multiple testing adjustment: For the adjustment of multiple testing of dose response relationship in 104 week mouse and rat studies, the FDA guidance for the carcinogenicity study design and data analysis suggests the use of test levels of α=0.005 for common tumors and α=0.025 for rare tumors for a submission with two species, and a significance level of α=0.01 for common tumors and α=0.05 for rare tumors for a submission with one species study in order to keep the false-positive rate at the nominal level of approximately 10%. A rare tumor is defined as one in which the published spontaneous tumor rate is less than 1%. For multiple pairwise comparisons of treated group with control the FDA guidance suggests the use of test levels α=0.01 for common tumors and α=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.

Since the present study is a 26 week study these rules are not applicable for the adjustment for multiple testing. With a conservative approach, in this reviewer's analysis all p-values were compared against α=0.05.

Reviewer's findings: Using the test level of α=0.05, this reviewer's analysis did not show statistically significant dose response relationship across vehicle control, low, medium , and high dose groups in the incidence of any of the observed tumor types in either sex. The pairwise comparisons also did not show statistically significant increased incidence of any of the observed tumor types in the low, medium, and high dose groups compared to the vehicle control group in either sex.

3. Evaluation of validity of the design of the study

Since, the tumor data analyses did not show statistically significant dose-response relationship or pairwise comparison in any of the tested tumor types in either sex, it is important to look into the validity of the design. For a transgenic mouse study using a positive control group, it is important to verify the performance of the positive control for the validation of the study. For a valid study the animals in the positive control group are expected to show significantly higher tumorogenicity compared to the animals in groups treated with the study compound group. Tables 4A and 4B show the results of the dose response relationship tests and the pairwise comparisons of tumor incidences using the data from positive control, low, medium, and high dose groups. The results show that the positive control group had statistically significant increased incidences of lungs adenoma, carcinoma, hemangiosarcoma and spleen hemangiosarcoma in both sexes of mice. The positive control group also had

statistically significant increased mortality compared to the treated groups.

The results indicate that the design of the study might be valid. However, other biological and toxic effects must be taken into consideration for the final evaluation.

4. Summary

In this submission the sponsor included a report of an animal carcinogenicity study in mice. This study was intended to assess the carcinogenic potential of UT-15C (Treprostinil Diethanolamine) in Tg.rasH2 mice when administered orally via gavage at appropriate drug levels for about 26 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.

Design: Two separate experiments were conducted, one in male and one in female mice. In each of these two experiments there were three treated groups, one vehicle control group (negative control), and one positive control group. A total of one hundred Tg.rasH2 mice of each sex were randomly assigned to treated and vehicle control groups in equal size of 25 animals per group. The positive control group had 15 mice in each sex. The dose levels for treated groups were 5.0, 10.0, and 20.0 mg/kg/day for male mice and 3.0, 7.5, and 15.0 mg/kg/day for female mice. The animals in the positive control group received a total of 3 urethane intraperitoneal injections on Study Days 1, 3, and 5. The animals in the vehicle control group received vehicle (sterile water for injection).

Results: The tests did not show statistically significant dose response relationship in mortality across vehicle control, low, medium, and high dose groups in either sex. The pairwise comparisons also did not show statistically significant increased mortality in the low, medium, and high dose groups compared to the vehicle control group in either sex.

The tests did not show statistically significant dose response relationship across vehicle control, low, medium, and high dose groups in the incidence of any of the observed tumor types in either sex. The pairwise comparisons also did not show statistically significant increased incidence of any of the observed tumor types in the low, medium, and high dose groups compared to the vehicle control group in either sex. The positive control group showed statistically significant increased mortality and incidences of lungs adenoma, carcinoma, hemangiosarcoma, and spleen hemangiosarcoma in both sexes of mice.

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5. Appendix

Table 1A: Intercurrent Mortality Rate
Male Mice

	Veh. Control		_____Low_____		____Medium___		_____High_____		_Pos. Control_	
	No. of		No. of		No. of		No. of		No. of	
Week	Death	Cum. %	Death	Cum. %	Death	Cum. %	Death	Cum. %	Death	Cum. %
Week 0 - 1	.	.	.	.	.	.	.	.	3	20
Week 11-20	.	.	.	.	.	.	.	.	4	47
Week 21-26	.	.	.	.	1	4	.	.	.	.
Ter. Sac.	25	100	25	100	24	96	25	100	8*	53
Total	N=25		N=25		N=25		N=25		N=15	

*** Animals in positive control were sacrificed on week 16**

Table 1B: Intercurrent Mortality Rate
Female Mice

	Veh. Control		_____Low_____		____Medium___		_____High_____		_Pos. Control_	
	No. of		No. of		No. of		No. of		No. of	
Week	Death	Cum. %	Death	Cum. %	Death	Cum. %	Death	Cum. %	Death	Cum. %
Week 0 - 1	.	.	.	.	1	4	.	.	1	7
Week 11-20	.	.	1	4	1	4	.	.	3	27
Week 21-26	.	.	.	.	.	.	1	4	.	.
Ter. Sac.	25	100	24	96	23	92	24	96	11*	73
Total	N=25		N=25		N=25		N=25		N=15	

*** Animals in positive control were sacrificed on week 16**

Table 2A: Intercurrent Mortality Comparison
Male Mice

Test	Statistic	P_Value*
Dose-Response	Likelihood Ratio	0.9866
Homogeneity	Log-Rank	0.3916

*** The p-values were calculated using data from the vehicle control, low, medium and high dose groups**

Table 2B: Intercurrent Mortality Comparison
Female Mice

Test	Statistic	P_Value*
Dose-Response	Likelihood Ratio	0.8950
Homogeneity	Log-Rank	0.5496

*** The p-values were calculated using data from the vehicle control, low, medium and high dose groups**

Table 3A: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons of Treated Groups with Vehicle Control in Male Mice

		0 mg	5 mg	10 mg	20 mg	P-Value			
		Veh. Cont.	Low	Med	High	Dose			
Organ Name	Tumor Name	N=25	N=25	N=25	N=25	Resp	VCvs L	VCvs.M	VCvs.H
cavity, nasal	adenocarcinoma	0	0	1	1	0.1869	.	0.5000	0.5000
ear	papilloma	0	0	1	0	0.5000	.	0.5000	.
harderian glands	adenoma	0	0	1	2	0.0606	.	0.5000	0.2449
lungs with bronchi	alveolar-bronchiolar adenoma	2	4	1	1	0.8020	0.3336	0.5000	0.5000
lungs with bronchi	adenoma+carcinoma	2	4	1	1	0.8020	0.3336	0.5000	0.5000
lung+spleen+testes	hemangiosarcoma	0	3	3	1	0.4643	0.1173	0.1173	0.5000
spleen	hemangiosarcoma	0	2	3	1	0.3817	0.2449	0.1173	0.5000
spleen+testes	hemangiosarcoma	0	3	3	1	0.4643	0.1173	0.1173	0.5000
stomach	papilloma	0	0	1	0	0.5000	.	0.5000	.
	squamous cell carcinoma	0	0	1	0	0.5000	.	0.5000	.
testes	hemangiosarcoma	0	1	0	0	0.5000	0.5000	.	.
thyroid glands	adenoma	1	0	0	0	0.7500	0.5000	0.5000	0.5000

VC=Vehicle Control

Table 3B: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons of Treated Groups with Vehicle Control in Female Mice

		0 mg	3.0mg	7.5mg	15.0mg	P-Value			
		Veh. Cont.	Low	Med	High	Dose			
Organ Name	Tumor Name	N=25	N=25	N=25	N=25	Resp	VCvs.L	VCvs.M	VCvs.H
cavity, nasal	adenocarcinoma	1	0	0	0	0.7423	0.4898	0.4792	0.5000
	carcinoma	0	0	1	0	0.5000	.	0.4898	.
	adenocarcinoma+carcinoma	1	0	1	0	0.6263	0.4898	0.7449	0.5000
ear	papilloma	0	1	0	0	0.4948	0.4898	.	.
harderian glands	adenoma	1	0	1	0	0.6239	0.4898	0.7340	0.5000
	carcinoma	0	0	0	1	0.2577	.	.	0.5000
	adenoma+carcinoma	1	0	1	1	0.4081	0.4898	0.7340	0.7551
lung	adenoma+carcinoma	2	2	2	2	0.4603	0.6798	0.6631	0.6954
lungs with bronchi	alveolar-bronchiolar adenoma	2	2	2	2	0.4603	0.6798	0.6631	0.6954
	mesothelioma	0	1	0	0	0.4898	0.5000	.	.
lungs+spleen+skin	hemangioma+hemangiosarcoma	2	1	0	3	0.2728	0.4844	0.7340	0.5000
salivary glands	mesothelioma	1	0	0	0	0.7423	0.4898	0.4792	0.5000
	sarcoma	0	1	0	0	0.4898	0.5000	.	.
skin (mammary area)	hemangioma	0	1	0	0	0.4948	0.4898	.	.
skin+spleen	hemangioma+hemangiosarcoma	2	1	0	3	0.2728	0.4844	0.7340	0.5000
spleen	hemangiosarcoma	2	0	0	3	0.1756	0.7449	0.7340	0.5000

VC=Vehicle Control

Table 4A: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons of Treated Groups and Vehicle Control with Positive Control in Male Mice

Organ Name	Tumor Name	Veh Cont. N=25	5 mg Low N=25	10 mg Med N=25	20 mg High N=25	Pos Cont. N=15	P_Value Dose Resp	PCvs.L	PCvs.M	PCvs.H	PCvs.VC
cavity, nasal	adenocarcinoma	0	0	1	1	0	0.2089	.	0.0741	0.0741	.
ear	papilloma	0	0	1	0	0	0.5098	.	0.0741	.	.
harderian glands	adenoma	0	0	1	2	0	0.0792	.	0.0741	0.1453	.
lungs with bronchi	alveolar-bronchiolar adenoma	2	4	1	1	15	<0.001	<0.001	<0.001	<0.001	<0.001
	alveolar-bronchiolar Carcinoma	0	0	0	0	4	<0.001	<0.001	<0.001	<0.001	<0.001
	alveolar-bronchiolar adenoma+carcinoma	2	4	1	1	15	<0.001	<0.001	<0.001	<0.001	<0.001
	hemangiosarcoma	0	0	0	0	3	<0.001	0.0025	0.0025	0.0025	0.0025
lung+spleen+testes	hemangiosarcoma	0	3	3	1	9	<0.001	<0.001	<0.001	<0.001	<0.001
spleen	hemangiosarcoma	0	2	3	1	9	<0.001	<0.001	<0.001	<0.001	<0.001
spleen+testes	hemangiosarcoma	0	3	3	1	9	<0.001	<0.001	<0.001	<0.001	<0.001
stomach	papilloma	0	0	1	0	0	0.5098	.	0.0741	.	.
	squamous cell carcinoma	0	0	1	0	0	0.5098	.	0.0741	.	.
testes	hemangiosarcoma	0	1	0	0	0	0.5098	0.0741	.	.	.
thyroid glands	adenoma	1	0	0	0	0	0.7549	.	.	.	0.0741

VC=Vehicle Control, PC=Positive Control

Table 4B: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons of Treated Groups and Vehicle Control with Positive Control in Female Mice

Organ Name	Tumor Name	Veh Cont. N=25	3 mg Low N=25	7.5mg Med N=25	15 mg High N=25	Pos Cont. N=15	P_Value Dose Resp	PCvs.L	PCvs.M	PCvs.H	PCvs.VC
cavity, nasal	adenocarcinoma	1	0	0	0	0	0.7500	.	.	.	0.1071
	carcinoma	0	0	1	0	0	0.5149	.	0.1111	.	.
cavity_nasal	adenocarcinoma+carci	1	0	1	0	0	0.6483	.	0.1111	.	0.1071
ear	papilloma	0	1	0	0	0	0.5100	0.1111	.	.	.
harderian glands	adenoma	1	0	1	0	0	0.6463	.	0.1154	.	0.1071
	carcinoma	0	0	0	1	0	0.2800	.	.	0.1071	.
	adenoma+carcinoma	1	0	1	1	0	0.5350	.	0.1154	0.1071	0.1071
lungs with bronchi	alveolar-bronchiolar adenoma	2	2	2	2	15	<0.001	<0.001	<0.001	<0.001	<0.001
	alveolar-bronchiolar carcinoma	0	0	0	0	10	<0.001	<0.001	<0.001	<0.001	<0.001
	alveolar-bronchiolar adenoma+carcinoma	2	2	2	2	15	<0.001	<0.001	<0.001	<0.001	<0.001
	hemangiosarcoma	0	0	0	0	2	0.0012	0.0159	0.0171	0.0148	0.0148
	mesothelioma	0	1	0	0	0	0.5050	0.1071	.	.	.
lungs+spleen+skin	hemangioma+hemangios	2	1	0	3	14	<0.001	<0.001	<0.001	<0.001	<0.001
salivary glands	mesothelioma	1	0	0	0	0	0.7500	.	.	.	0.1071
	sarcoma	0	1	0	0	0	0.5050	0.1071	.	.	.
skin (mammary area	hemangioma	0	1	0	0	0	0.5100	0.1111	.	.	.
skin+spleen	hemangioma+hemangios	2	1	0	3	14	<0.001	<0.001	<0.001	<0.001	<0.001
spleen	hemangiosarcoma	2	0	0	3	14	<0.001	<0.001	<0.001	<0.001	<0.001

VC=Vehicle Control, PC=Positive Control

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

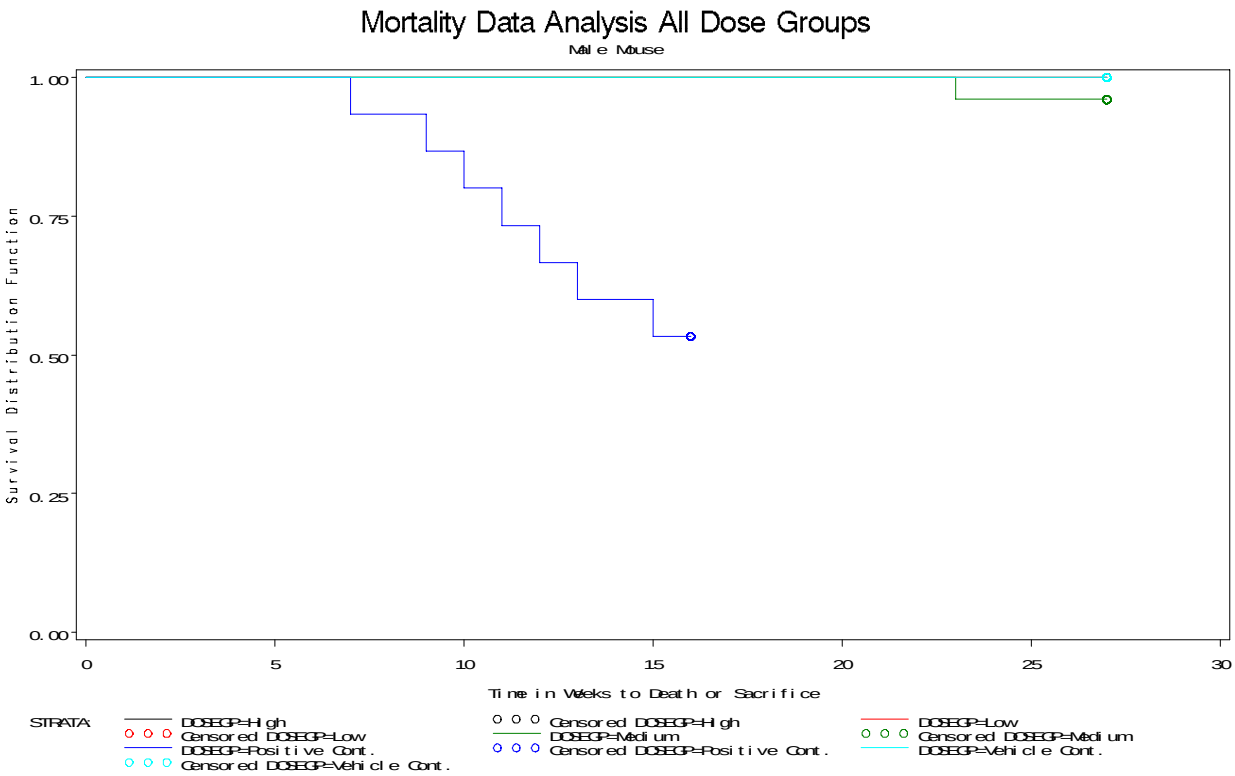
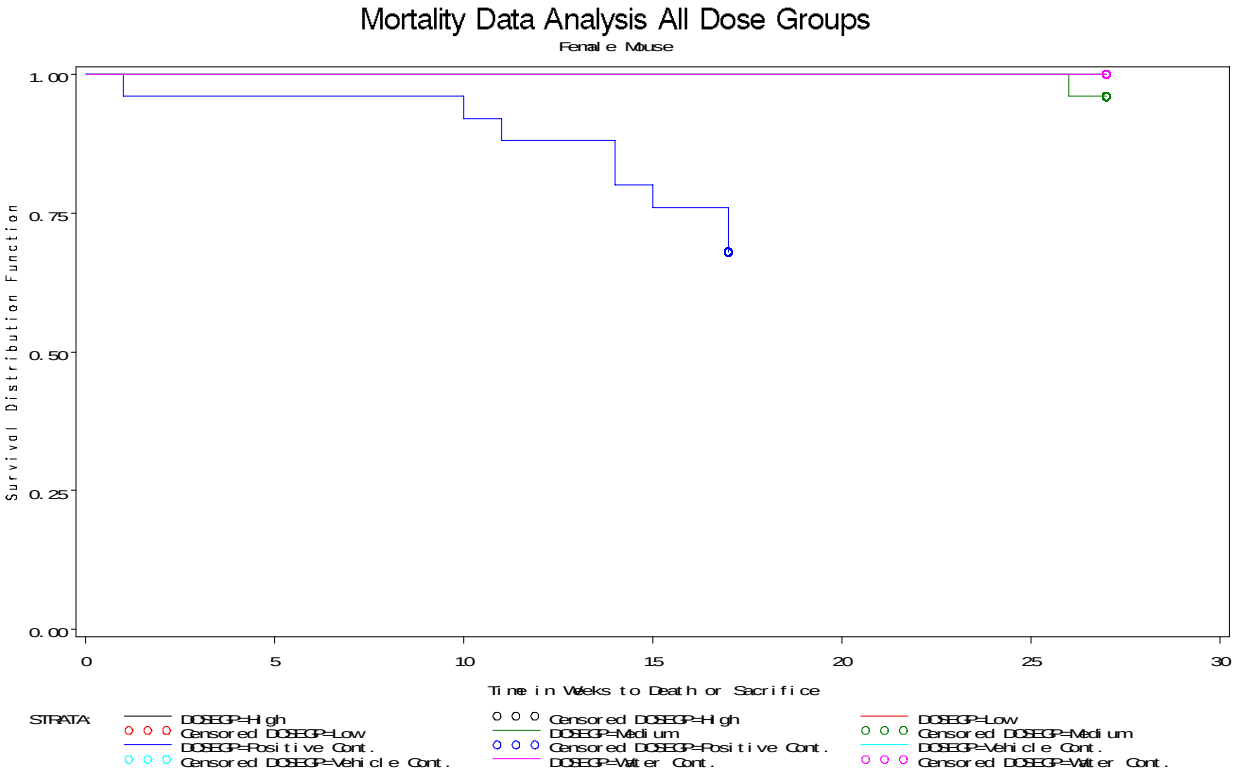


Figure 1B: Kaplan-Meier Survival Functions for Female Mice



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/s/

MOHAMMAD A RAHMAN
05/31/2012

KARL K LIN
06/01/2012
Concur with review

STATISTICS FILING CHECKLIST FOR A NEW NDA/BLA

NDA Number: 203496 **Applicant: United Therapeutics** **Stamp Date:** 12/27/11

Drug Name: treprostinil diethanolamine **NDA/BLA Type:**

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

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

IS THE STATISTICAL SECTION OF THE APPLICATION FILEABLE? __Yes_____

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

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

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

File name: 5_Statistics Filing Checklist for a New NDA_BLA110207

STATISTICS FILING CHECKLIST FOR A NEW NDA/BLA

John Lawrence	2-15-2012
Reviewing Statistician	Date
Jim Hung	2-15-2012
Supervisor/Team Leader	Date

File name: 5_Statistics Filing Checklist for a New NDA_BLA110207

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/s/

JOHN P LAWRENCE
02/15/2012

HSIEN MING J HUNG
02/15/2012