

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 20-829

STATISTICAL REVIEW(S)

Statistical Review and Evaluation

NDA #: 20-829 and 20-830
Applicant: Merck
Name of Drug: Singulair (Montelukast Sodium) 10mg
Tablets and 5mg Chewable Tablets
Indication: Treatment of Symptoms of Asthma
Documents Reviewed: Volumes 1.1,1.2,1.96-1.114 of NDA 20-830
dated February 21, 1997

AUG 16 1997

This review pertains to 3 studies in the treatment of asthma, two in adults (Studies 20 and 31) and the other in children 6 to 14 years old (Study 49); 2 studies in exercise induced asthma, one in adults (Study 42) and the other in children (Study 40) ; and one corticosteroid sparing trial in adults (Study 46). The 5mg montelukast chewable tablets were used in the studies in children. The study reports were presented in both submissions and, therefore, only the jackets of one submission were reviewed.

The medical officer of this submission is P. Honig, M.D. (HFD-570), with whom this review was discussed.

This review will mainly focus on the primary efficacy variables. The results of the secondary efficacy variables will be mentioned briefly to highlight the consistency of efficacy.

Methods of analyses were discussed in the sponsor's data analysis plans. The sponsor followed these plans in their study reports.

I. Study 20

A. Study Description and Method of Analysis

This study was an international multi-center, randomized, double blind, parallel group study in nonsmoking asthmatic patients 15 years of age or over with a FEV₁ between 50 and 85% of predicted normal and demonstrating reversibility of at least 15% with beta-agonist. Up to 25% of the patients were allowed concomitant use of theophylline.

There was a 2-week placebo run-in period, a 12-week treatment period and, for a subset of the patients, a 3-week placebo wash-out period. (Other non-placebo patients could go into a 9-month double-blind extension.) The purpose of the placebo washout period was to see how Montelukast patients responded when taken off drug. Patients during the placebo run-in period had to have a predetermined level of daytime symptoms (biweekly total score of at least 64) and daytime and nighttime beta-agonist use (weekly

average of at least one puff per day).

Clinic visits were every three weeks during the 12 week treatment period. An additional clinic visit was scheduled after three weeks for those patients who went into the placebo washout period. Spirometry measurements were obtained between 6 and 9 AM of each visit, approximately 8 to 10 hours after the previous bedtime dose.

Four daytime asthma symptom scores were assessed, at bedtime and before taking medication, on 7 point scales:

- How often did you experience asthma symptoms today?
0 1 2 3 4 5 6
None of the time All of the time
- How much did your asthma symptoms bother you today?
0 1 2 3 4 5 6
Not at all bothered Severely bothered
- How much activity could you do today?
0 1 2 3 4 5 6
More than usual activity Less than usual activity
- How often did your asthma affect your activity today?
0 1 2 3 4 5 6
None of the time All of the time

The daily daytime symptom score was determined by averaging the daily scores for the four questions. The average daytime symptom score for the visit was determined by averaging the daily symptom scores over all days between two consecutive visits.

Randomization was done by stratified randomization in each center. The two strata were theophylline users and non-users. Blocked randomization was used with a block size of 7 (three montelukast patients and two of both placebo and beclomethasone.) Patients without concurrent theophylline use were assigned the smallest patient numbers, while patients with concurrent theophylline were assigned the largest patient number available.

The primary efficacy variables were daytime asthma symptom scores and FEV₁ both averaged over the whole treatment period. Both efficacy variables had to be significant to declare efficacy. The primary efficacy variables were analyzed by an analysis of

variance with factors: treatments, centers and strata (theophylline users or non-users). Treatment-by-center and treatment-by-stratum interaction were tested by supplementary analyses.

B. Results

Eight hundred and ninety-five patients (257 placebo, 387 montelukast, and 251 beclomethasone) were randomized at 38 centers in 19 countries. About 10% of the patients were taking theophylline.

The 15 patients of study center 020-030 were not included in the intent-to-treat analyses because of Good Clinical Practice compliance issues. [This reviewer reran the primary analyses including this center. The exclusion of this center had negligible effect on the results of the study.] A further 10 patients were excluded from the intent-to-treat analysis of FEV₁ and 19 patients from the intent-to-treat analysis of daytime asthma symptom score because they either did not have baseline scores or on-treatment data.

The treatment groups were comparable at baseline in demographic and baseline efficacy variables.

Table 1 contains the percent changes from baseline for FEV₁ and p-values comparing treatments (average over the treatment period). Table 2 contains the mean average changes in daytime asthma symptom scores and the p-values comparing treatments. Montelukast was significantly better than placebo but less effective than beclomethasone for these parameters.

Significant results for both efficacy variables, not shown here, were also seen at the last on-treatment clinic visit.

Significance of montelukast over placebo and beclomethasone over montelukast were seen in most secondary efficacy variables, global evaluations and quality of life assessments.

The treatment-by-center and treatment-by-stratum interactions were not significant ($P > 0.05$) for both primary efficacy variables. The treatment-by-gender interaction was also not-significant for these variables.

C. Reviewer's Comments

This study showed efficacy of Montelukast in adults.

II. Study Protocol 31

A. Study Description and Method of Analysis

This study was similar to study 20 with the following exceptions. It did not contain Beclomethasone. Up to 25% of the patients were allowed concomitant use of inhaled corticosteroids rather than theophylline. Randomization was by blocked randomization in each center with a block size of ten (6 montelukast patients and 4 placebo patients).

B. Results

There were 681 randomized patients (273 placebo and 408 montelukast) at 52 U.S. centers who entered the study. About 23% of the patients were taking inhaled corticosteroids.

All randomized patients (N=2, one in each group) from center 031-028 were excluded from the intent-to-treat analyses because case report forms could not be verified (the center lost their copies and all source documents). These patients are not included in the 681 patients listed above. A further 5 patients were excluded from the intent-to-treat analysis of FEV₁ and 8 patients from the intent-to-treat analysis of daytime asthma symptom score because they either did not have baseline scores or on-treatment data.

The treatment groups were comparable at baseline in demographic and baseline efficacy variables.

Table 3 contains the average percent changes from baseline for FEV₁ over the whole treatment period and p-values comparing treatments. Table 4 contains the mean changes in daytime asthma symptom scores over the whole treatment period and the p-values comparing treatments. Montelukast was significantly better than placebo for these primary efficacy parameters.

Significant results for both efficacy variables, not shown here, were also seen at the last on-treatment clinic visit.

Significance of montelukast over placebo were seen in most secondary efficacy variables, global evaluations and quality of life assessments.

The treatment-by-center interaction was not significant ($P > 0.05$) for both primary efficacy variables. The treatment-by stratum interaction was significant for daytime symptom score. The patients on corticosteroids showed only a small difference between treatments with a change of -0.24 for placebo and -0.29 for montelukast. Both users of corticosteroids and non-users showed comparable increases in FEV₁, however. The treatment-by-gender interaction was significant for FEV₁. Here the interaction

was a quantitative interaction with more increase over placebo in males 9.5% than females 7.2%.

C. Reviewer's Comments

This study showed efficacy in adults. If a patient is taking corticosteroid, efficacy might be limited to FEV₁, no effect in daytime asthma systems was demonstrated.

III. Study Protocol 49

A. Study Description and Method of Analysis

This study was similar to study 20 with the following exceptions. It was in children 6- to 14- years of age rather than adults. It was only 8 weeks rather than 12 weeks. This study used the 5-mg chewable tablets rather than the 10-mg tablets used with adults. Up to 40% of the children were allowed to continue on inhaled corticosteroids. The stratification factor was therefore corticosteroids use or non-use. The primary efficacy variable was defined to be FEV₁ only rather than both FEV₁ and daytime asthma score.

The daytime asthma score was defined differently also. The patient answered each of the following questions (based on symptoms since arising) by circling the most appropriate number:

- How much of the time did you have trouble breathing today?
None of the time A little of the time Some of the time A good bit of the time Most of the time All of the time
0 1 2 3 4 5

- How much did your asthma bother you today?
Did not bother me Bothered me a little Bothered me somewhat Bothered me a good deal Bothered me very much Bothered me as much as possible
0 1 2 3 4 5

- How much of the time did your asthma limit your activity today?
None of the time A little of the time Some of the time A good bit of the time Most of the time All of the time
0 1 2 3 4 5

B. Results

There were 336 patients (135 placebo and 201 montelukast) randomized into the trial. About 37% of the patients were on inhaled corticosteroids.

The treatment groups were comparable at baseline in demographic and baseline efficacy variables.

Five patients (2 placebo and 3 Montelukast) from center 049-032 were excluded because of significant deviations from good clinical practice. An additional 4 patients were excluded from the analysis of FEV₁ and an additional two patients from the analysis of asthma symptom scores because they either did not have baseline scores or on-treatment data.

Table 5 contains the percent changes from baseline for average FEV₁ and p-values comparing treatments. Montelukast was significantly better than placebo for this primary efficacy parameters. Table 6 contains the mean changes in average daytime asthma symptom scores and the p-values comparing treatments for this analysis. This difference was not significant. It should be emphasized that this was not a primary efficacy parameter in this study.

C. Reviewer's Comments

The evidence for efficacy is weaker here than in the adult studies. Since the FEV₁ measurements are at about 8 to 10 hours after dosing while the daytime asthma scores are at near the end of dosing interval, no end of dosing interval efficacy is demonstrated here. Less efficacy was seen in daytime asthma score in inhaled corticosteroid users (placebo mean change -0.11, Montelukast mean change -0.14) than in nonusers (placebo mean change -0.13, Montelukast mean change -0.22). Since the proportion of inhaled corticosteroid users was higher in this study than in the adult study (Study 31), this also may have caused the lack of overall efficacy in this parameter. [The daytime asthma scores are not equivalently defined, however.]

Some efficacy was seen in secondary measures: total daily b-agonist use and clinic assessed AM PEFr but not in nocturnal assessments and patient assessed AM PEFr.

IV. Study 42 - Exercise Induced Asthma

A. Study Design and Method of Analysis

This was a multi-center, placebo controlled, randomized, double blind, parallel group exercise challenge study with a one week

placebo run-in period, a 12 week treatment period, and a two week placebo washout period.

Two exercise challenges were held during the placebo run-in period. The patient had to demonstrate a post-exercise fall of at least 20% at both challenges. Exercise challenges were also done at weeks 4, 8 and 12 of treatment and after 2 weeks of placebo washout. The exercise challenge after two weeks of placebo washout was to test for persistence of effect.

The exercise challenge had a two minute or more warm up to obtain a targeted heart rate of 80 to 90% of age predicted maximum. This targeted heart rate was maintained for 6 minutes.

Spirometry was performed immediately after exercise and at 5, 10, 15, 30, 45 and 60 minutes. If by 60 minutes the patient had not returned to within 5% of the pre-exercise level, an FEV₁ measurement was obtained at 75 minutes, and, if necessary, at 90 minutes. If the patient had still not returned to within 5% of the pre-exercise FEV₁, then rescue beta-agonist was given.

The primary efficacy variables in this study were AUC_{0-60min} and Maximum Percent Fall in FEV₁. The sponsor considered AUC_{0-60min} primary, while the medical officer considered Maximum Percent Fall in FEV₁ most important.

The primary analyses was endpoint changes from baseline with last value carried forward. To calculate AUC_{0-60min} the last spirometry value at the clinic assessment was also carried forward.

The AUC_{0-60min} was calculated as area below the pre-exercise FEV₁. If the FEV₁ went above pre-exercise FEV₁, no positive area was added.

The primary endpoints were analyzed by an analysis of variance with factors treatment and center. Treatment-by-center interaction was assessed in supplementary analyses.

The sponsor also analyzed AUC_{0-60min} and Maximum Percent Fall in FEV₁ with a repeated measures (Weeks 4, 8 and 12) mixed model.

B. Results

There were 110 patients (56 placebo and 54 montelukast) who entered the trial. The treatment groups were comparable at baseline in demographic and baseline efficacy variables.

Four patients (two in each treatment group) were excluded from the intent-to-treat analysis of the primary efficacy variables because they either had no baseline values or no on-treatment values and hence no changes from baseline could be obtained.

The table below shows the mean changes from baseline for the week 12 endpoint analysis of AUC_{0-60min} of FEV₁. Montelukast showed significantly less decrease than placebo in the hour after exercise.

Analysis of AUC_{0-60min} of FEV₁ (week 12 endpoint)
(Intent-to-treat)

Treatment	N	Mean(%*min) Change from baseline at week 12			
		Baseline	Mean	SD	P-value
Placebo	54	1540.0	-99.2	983.4	0.001
Montelukast	52	1397.6	-630.0	783.1	

The table below shows the mean changes from baseline for the week 12 endpoint analysis of maximum percent fall in FEV₁. Montelukast showed significantly less of a fall in FEV₁ than placebo after exercise.

Maximum Percent Fall in FEV₁ (Week 12 endpoint)
(Intent-to-treat)

Treatment	N	Mean (%) Change from baseline at week 12			
		Baseline	Mean	SD	P-value
Placebo	54	38.3	-5.90	14.61	0.003
Montelukast	52	36.45	-14.12	12.56	

The repeated measures analysis found no difference between the slope of the two treatments but a difference in intercept for both primary endpoints. The slope for both treatments looked to be zero, which means that the treatment difference at weeks 4, 8 and 12 were effectively constant and significant.

Fifty percent (26/52) of Montelukast patients were protected against a 20% drop in FEV₁ compared to 37% (20/54) of the placebo patients. This difference is not significant (p=0.177, binomial test).

Two weeks after cessation of treatment the montelukast parameter values approached the placebo values but did not exceed them. The

protection has worn off by two weeks after treatment.

C. Reviewer's Comments

This study showed an effect on AUC FEV₁ and max percent fall in FEV₁ but only 50% of the patients were protected against a 20% fall in FEV₁ on Montelukast. Whether such a protection percentage is adequate must be left to clinical judgement.

V. Study 040 - Exercise Induced Asthma

A. Study Design and Method of Analysis

This was a two period, randomized, double-blind, crossover exercise challenge study comparing montelukast 5-mg chewable tablet with placebo in children 6 to 14 years of age. There was a three day treatment period with the exercise challenge at the end of the third day. The exercise challenge was done 20 to 24 hours post-dose. There was a 4-day washout period between treatments.

Children were exercised on a treadmill for 6 minutes at a workload calculated to increase the patient's heart rate to approximately 160 to 190 beats per minute. This workload was used on all exercise challenges for that patient.

AUC_{0-60 min} and Maximum FEV₁ percent fall from pre-exercise challenge FEV₁ were analyzed by an analysis of variance with factors for centers, sequence, subjects within center-by-sequence, period and treatment.

B. Results

There were 27 children who entered the study. Two patients on placebo during the second period dropped out and did not perform an exercise challenge. Therefore the primary efficacy analyses included only 25 patients who took both treatments.

The table below provides the treatment means and p-values comparing treatments for the primary efficacy variables. Montelukast provided more protection against fall in FEV₁ than placebo.

Variable	Placebo Mean(SD) n=25	Montelukast Mean(SD) n=25	P-value
AUC _{0-60 min} FEV ₁ (%*min)	-589.72 (705.27)	-264.60 (271.56)	0.013
Maximum % Fall	-26.11 (13.93)	-18.27 (12.54)	0.009

Sixty percent of the children were protected against a 20% drop in FEV₁ on Montelukast compared to only 40% while on placebo. This difference is not significant using McNemar's test.

No period or carryover effects were detected ($P > 0.05$).

C. Reviewer's Comments

This study showed an effect on AUC FEV₁ and max percent fall in FEV₁ but only 60% of the patients were protected against a 20% fall in FEV₁ on Montelukast. Whether such a protection percentage is adequate must be left to clinical judgement.

VI. Study 046 - Corticosteroid Sparing Study

A. Study Description and Method of Analysis.

This was a high-dose inhaled corticosteroid study to investigate the ability of Montelukast to allow tapering of inhaled corticosteroids in asthmatic patients. It was a multi-center, double-blind, randomized, parallel group study with a one month single-blind placebo period where patients were tapered once or twice (at two week intervals) while maintaining FEV₁ at 90% or greater of their run-in baseline value (pre-study visit and visit 1 average). If the FEV₁ fell below 90%, the inhaled cortico-steroid was increased. The purpose of this run-in period was to handle the situation that the dose of corticosteroids that the patient was using might be higher than the patient needed to control his asthma.

Patients entered a pre-randomization baseline period during which baseline values of FEV₁, daytime symptom score and total daily inhaled beta-agonist use were determined. These three parameters were used to determine whether the patient's inhaled cortico-steroid dose would be tapered during the double-blind period.

The patients who entered the study were stratified into high and low dose groups with separate randomizations in each group within a center.

The inhaled corticosteroid tapering criteria depended upon a composite clinical score determined over the clinic visit for FEV₁ or the last 7 days for the two diary components. If pre-beta-agonist FEV₁ $\geq 90\%$ of pre-randomized baseline then 1 point was scored. If daytime symptom score $\leq 120\%$ of pre-randomized baseline, another point was added. If beta-agonist use $\leq 135\%$ of pre-randomized baseline, another point was added. If the composite score was 3, inhaled corticosteroid was tapered. If the composite score was 2, the dose was maintained. If the composite score was 0 or 1, the dose of corticosteroid was increased. The taper dose or dose increase in puffs/day were proportional to the

dosage of the inhaled corticosteroids in puffs per day that the patient was currently taking.

The primary efficacy variable was last dose of inhaled corticosteroid as a percent change from pre-randomized baseline dose. Since the patients were using a variety of inhaled corticosteroids, this variable is independent of the dosage of corticosteroid. (It is also why the dose increase or dose taper were proportional to the current dose taken.) This percent was analyzed by an analysis with factors for treatment, stratum and center. The treatment-by-stratum and treatment-by-center interactions were assessed in supplementary analysis and found to be not significant.

B. Results

The table below provides the mean percent changes in last tolerated dose of inhaled corticosteroids and p-value comparing treatments. Montelukast was able to reduce the inhaled corticosteroid dosages significantly more than placebo.

Percent Change from baseline Last Tolerated dose of inhaled corticosteroids (Intent-to-treat)

Treatment	N	Mean (mcg/day)	Percent Change from pre-randomized baseline		
		Baseline	Mean	SD	P-value
Placebo	113	1078.8	30.27	67.37	0.046
Montelukast	112	975.9	46.73	62.22	

C. Reviewer's Comments

This study demonstrated that Montelukast would provide some steroid tapering.

The tapering criteria allowed a patient to be slightly worse and still have the dosage of inhaled corticosteroid reduced. This may partially explain why the placebo patients were able to further reduce their inhaled corticosteroid from their baseline level even with the run-in tapering period.

VII. Overall Conclusions

Studies 20 and 31 showed efficacy of Montelukast in adults in AUC FEV₁ and daytime asthma score averaged over the treatment period.

Study 49 showed efficacy for AUC FEV₁ in children 6- to 14- years of age.

Both studies 31 and 49 showed almost no efficacy in daytime symptom score if patients were taking corticosteroid. This difference was not seen in AUC FEV₁. Both corticosteroid users and non-users increased their AUC FEV₁.

Both exercise challenge studies (Studies 40 and 42) showed efficacy for AUC FEV₁ and Maximum percent fall in FEV₁. However, only 50 to 60% of the patients were protected against a fall of 20% in FEV₁.

Montelukast showed steroid sparing ability in Study 46 where the mean reduction from baseline of corticosteroid dosage was 47% for Montelukast and 30% for placebo.

/S/

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Mathematical Statistician HFD-715

Concur: Dr. Wilson

/S/ 8/8/97

Dr. Nevius

/S/ 8/16/97

This review contains 12 pages of text and 6 pages of tables.

cc:
Orig NDA 20-829
NDA 20-830
HFD-570
HFD-570/Dr. Honig
HFD-570/Ms. Trout
HFD-715/Div. File
HFD-715/Dr. Gebert
HFD-715/Dr. Wilson

Table 1

Analysis of FEV1

Study 20

(Intention-To-Treat Approach)

Treatment	N	Mean (L)		Percent Change From Baseline			
		Baseline	Treatment Period	Mean	SD	LS Mean	95% CI for Mean
Placebo	249	2.21	2.23	1.07	15.87	0.71	(-2.27, 3.69)
Montelukast	375	2.16	2.32	7.49	17.01	7.35	(4.61, 10.08)
Beclomethasone	246	2.10	2.38	13.30	19.72	13.12	(10.06, 16.18)

Comparison Between Treatments		p-Value	LS Mean	95% CI for Difference
Montelukast vs Placebo		<0.001	6.64	(3.89, 9.38)
Beclomethasone vs Placebo		<0.001	12.41	(9.39, 15.44)
Montelukast vs Beclomethasone		<0.001	-5.78	(-8.53, -3.02)

p-Value For Effect

Treatment	<0.001
Study center	<0.001
Stratum	0.751

Root MSE of Percent Change = 17.02

Table 2
 Analysis of Daytime Symptom Score
 Study 20
 (Intention-To-Treat Approach)

Treatment	N	Mean (Score)		Change From Baseline			
		Baseline	Treatment Period	Mean	SD	LS Mean	95% CI for Mean
Placebo	245	2.40	2.14	-0.26	0.74	-0.17	(-0.30, -0.05)
Montelukast	372	2.35	1.85	-0.49	0.81	-0.41	(-0.53, -0.29)
Beclomethasone	244	2.38	1.68	-0.70	0.80	-0.62	(-0.75, -0.49)
Comparison Between Treatments		p-Value		LS Mean		95% CI for Difference	
Montelukast vs Placebo		<0.001		-0.24		(-0.35, -0.12)	
Beclomethasone vs Placebo		<0.001		-0.44		(-0.57, -0.31)	
Montelukast vs Beclomethasone		<0.001		0.21		(0.09, 0.33)	
p-Value For Effect							
Treatment		<0.001					
Study center		<0.001					
Stratum		0.410					
Root MSE of Change = 0.73							

Table 3

Study 31

Analysis of FEV1

(Intention-To-Treat Approach)

Treatment	N	Mean (L)		Percent Change From Baseline			
		Baseline	Treatment Period	Mean	SD	LS Mean	95% CI for Mean
Placebo	270	2.54	2.64	4.22	12.67	3.21	(1.45, 4.96)
Montelukast	406	2.47	2.78	13.05	13.84	12.10	(10.60, 13.61)

Comparison Between Treatments		p-Value	LS Mean	95% CI for Difference
Montelukast vs Placebo		<0.001	8.90	(6.84, 10.96)

p-Value For Effect	
Treatment	<0.001
Study center	0.359
Stratum	0.012

Root MSE of Percent Change = 13.28

Table 4
 Analysis of Daytime Symptom Score
 Study 31
 (Intention-To-Treat Approach)

Treatment	N	Mean (Score)		Change From Baseline			
		Baseline	Treatment Period	Mean	SD	LS Mean	95% CI for Mean
Placebo	269	2.49	2.32	-0.18	0.59	-0.17	(-0.25, -0.08)
Montelukast	404	2.51	2.10	-0.41	0.69	-0.39	(-0.47, -0.32)
Comparison Between Treatments							
Montelukast vs Placebo				p-Value	LS Mean	95% CI for Difference	
				<0.001	-0.23	(-0.33, -0.13)	
p-Value For Effect							
Treatment	<0.001						
Study center	0.119						
Stratum	0.357						
Root MSE of Change = 0.65							

Table 5
Analysis of FEV₁

Study 49
(Intention-To-Treat Approach)

Treatment	N	Mean (L)			% Change From Baseline		
		Baseline	Treatment Period	Mean	SD	LS Mean	95% CI for Mean
Placebo	131	1.85	1.93	4.16	10.74	3.58	(1.29, 5.87)
Montelukast	196	1.85	2.01	8.71	12.54	8.23	(6.33, 10.13)
Comparison Between Treatments							
Montelukast vs Placebo				p-Value	LS Mean	95% CI for Difference	
				<0.001	4.65	(1.92, 7.38)	
p-Value For Effect							
Treatment	<0.001						
Study center	0.849						
Stratum	0.370						
Root MSE of % Change = 12.05							

Table 6
 Analysis of Daytime Symptom Score
 Study 49
 (Intention-To-Treat Approach)

Treatment	N	Mean (Score)		Change From Baseline			
		Baseline	Treatment Period	Mean	SD	LS Mean	95% CI for Mean
Placebo	132	1.26	1.14	-0.12	0.55	-0.09	(-0.19, 0.02)
Montelukast	197	1.28	1.09	-0.19	0.58	-0.16	(-0.25, -0.07)
Comparison Between Treatments							
Montelukast vs Placebo			p-Value		LS Mean		95% CI for Difference
			0.273		-0.07		(-0.20, 0.06)
p-Value For Effect							
Treatment			0.273				
Study center			0.714				
Stratum			0.265				
Root MSE of Change = 0.57							

**STATISTICAL REVIEW AND EVALUATION
STABILITY STUDY**

NDA Number: 20-830 and 20-829
Applicant: Merck
Name of Drug: Singulair® Chewable Tablets and Singulair® Tablets
Statistical Reviewer: Girish Aras Ph. D. (HFD-715)
Chemistry Reviewer: John Leak Ph. D. (HFD-570)
Document Reviewed: Stability Report, dated March 18, October 29, November 26 and December 4, 97
Date of Consult: October 6, 97

Date: DEC 15 1997

I. Introduction

The sponsor submitted 18 and 12 months of stability data for 3 developmental batches (MR-3230, MR-3239 and MR-3251) on March 19, 97 for bottles and blisters, respectively, for 10 mg tablets and 18 months of stability data for bottles and blisters for 5 mg tablets on a 3.5" diskette for Singulair® Chewable Tablets and Singulair® Tablets stored at :
The data were also submitted in the document referenced above. Based on their analyses, the sponsor has proposed expiration periods of 24 and 12 months for bottles and blisters, respectively, for 10 mg Singulair® Chewable Tablets and 24 months for bottles and blisters, respectively, for 5 mg Singulair® Tablets.

The sponsor's data described above is on three developmental batches only. The sponsor recently submitted 6 month data (November 26, 97) for one commercial batch MR-3339.

II. Stability Parameters

The following list of stability parameters with specification was used to evaluate the stability for 10 mg Singulair® Chewable Tablets and 5 mg Singulair® Tablets.

Table 1. Specifications

III. Reviewer's Analyses

The reviewer analyzed the data submitted by the sponsor on three developmental batches using the FDA stability program. The data from the commercial batch are not adequate to perform a valid statistical analysis. In addition, according to chemistry reviewer, these data cannot be combined with the data from the developmental batches. The conditions under which they were produced are different. Hence only the developmental batches were analyzed for this review. The FDA recommended test schedule is to test the product every 3 months during the first year, and every 6 months, thereafter. However, this schedule was not followed for some of the parameters as described in the remarks for the tables below. As there was only one data point for the HDPE bottle the sponsor has not submitted adequate data for a statistical analysis. The reader is cautioned that the statistical methods used for prediction beyond the testing period are valid only under the assumptions that the conditions of the experiment remain unchanged and linearity of the fitted equation holds for that period.

The predicted expiry given in tables below are not necessarily due to crossing of the 95% confidence band with the specification limits, but could be due to Biometrics program's convention of not extrapolating maximum predicted expiry beyond 4 times the study period. As mentioned before, the tables are based solely on the data from the developmental batches.

The data from the commercial batch, though far from adequate, falls inside the prescribed specification limits. By inspection, the individual values do not appear to differ from the developmental batches, though occasional differences can be noted, perhaps due to higher initial values for some variables in the commercial batches. However, even these entries are well below the specification limits. A statistical judgment on prediction and extrapolation based on commercial batches has to be delayed till adequate data is generated on at least 3 batches for 12 or 18 months.

Table 2. Statistical Summary for Stability Batches of 10 mg Singulair® Chewable Tablets

Packaging Type	Analysis Parameter	Model*	Least Favorable Batch**	Predicted Expiry (Months)
14ozHDPE	Degradation Products Sulfoxide Cis-isomer Dissolution Moisture	Common Slope Not Combined Combined Not Combined	MR-3230	
30mlHDPE	Degradation Products Sulfoxide Cis-isomer Dissolution Moisture	Common Slope Not Combined Common Slope Combined		
75HDPE90	Degradation Products Sulfoxide Cis-isomer Dissolution Moisture	Common Slope Not Combined Combined Not Combined	MR-3230	
75HDPE30	Degradation Products Sulfoxide Cis-isomer Dissolution Moisture	Combined Combined Common Slope Common Slope	MR-3239	
Blisters	Degradation Products Sulfoxide Cis-isomer Dissolution Moisture	Combined Combined Common Slope Not Combined		

* Models:
 Combined = Common slopes and common intercepts
 Common Slopes = Common slopes but separate intercepts
 Not Combined = Separate slopes and separate intercepts

** Least Favorable Batch = Stability Batch with the shortest predicted expiry
 NA = Not Applicable

- Data available only at 6 month-intervals and not at 3 month-intervals during first year, as requested in the FDA guideline.

Table 3. Statistical Summary for Stability Batches of 5 mg Singulair® Tablets

Packaging Type	Analysis Parameter	Model*	Least Favorable Batch**	Predicted Expiry (Months)
14ozHDPE	Degradation Products	-	-	-
	Sulfoxide	Combined	NA	
	Cis-isomer	Not Combined	NA	
	Dissolution Moisture	Not Combined Combined	MR-3276	
30mlHDPE	Degradation Products	-	-	-
	Sulfoxide	Combined	NA	
	Cis-isomer	Not Combined	NA	
	Dissolution Moisture	Not Combined Common Slope	MR-3276	
75HDPE90	Degradation Products	-	-	-
	Sulfoxide	Combined	NA	
	Cis-isomer	Not Combined	NA	
	Dissolution Moisture	Not Combined Combined	MR-3276	
75HDPE30	Degradation Products	-	-	-
	Sulfoxide	Combined	NA	
	Cis-isomer	Not Combined	NA	
	Dissolution Moisture	Common Slope Combined	MR-3276	
Blister	Degradation Products	-	-	-
	Sulfoxide	Combined		
	Cis-isomer	Combined		
	Dissolution Moisture	Common Slope Combined		

* Models:
 Combined = Common slopes and common intercepts
 Common Slopes = Common slopes but separate intercepts
 Not Combined = Separate slopes and separate intercepts

** Least Favorable Batch = Stability Batch with the shortest predicted expiry
 NA = Not Applicable

- Data available only at 6 month-intervals and not at 3 month-intervals during first year, as requested in the FDA guideline.

IV. Conclusion

Given the acceptability of using the developmental batches for the assessment of stability, the overall stability data support expiry dates proposed by the sponsor for 75mL HDPE bottles, 90 and 30 tablet count. They are 24 months for 10 mg and 5 mg formulations in bottles. Package types, 14oz. HDPE and 30 mL (4 tablets) HDPE bottles support similar expiry periods, however there are no data at 3 and 9 months. The package type HPPE bottle does not have adequate data points to support extrapolation beyond the period of experiment, 12 months.

The data for Blister packaging support the sponsor's proposed expiry dates. They are 12 and 24 months for 10 mg and 5 mg blisters, respectively.

Extrapolation beyond the testing period is based on the assumptions that the condition of the experiment remains unchanged and the linearity of the fitted equation holds for that period.

|S|

Girish Aras, PhD

Concur: Dr. Karl Lin

|S| 2/15/97

cc:

Orig. NDA 20-829 and 20-830

HFD-570 / Division File

HFD-570 / JLeak

HFD-715 / Division File, Chron

HFD-715 / GARas, KLin

STATISTICAL REVIEW AND EVALUATION CARCINOGENICITY

IND #:

Date:

AUG 12 1996

Applicant: Merck Research Laboratories

Name of Drug: Montelukast Sodium

Documents Reviewed: 2-29-96 Vol 35.20-35.22

5-22-96 Supporting Statistical Analysis Datasets & Documentation

Statistical Reviewer: B Bono, M.S.

Pharmacologist: S Williams, Ph.D.

Key Words: Peto, trend test, adjusted p -values, adjusted α -levels

Text in italics is from the Investigational New Drug Application submitted by the sponsor.

Summary of Review

- There are no statistically significant p -values from the trend test in either of the two animal studies provided that:
 - the α -level of a "rare" tumor is 0.025 and the α -level of a "common" tumor is 0.005, and
 - a pancreatic islet adenoma is a "common" tumor among rats.
- The pairwise comparisons of the control with the low and high dose groups for hepatocellular carcinoma liver tumors among male rats is not statistically significant provided that:
 - the α -level of a "common" tumor is 0.01, and
 - a hepatocellular carcinoma liver tumor is "common" among rats.
- The pairwise comparisons of the control with the middle and high dose groups for pancreatic islet adenoma tumors among male rats is not statistically significant provided that:
 - the α -level of a "common" tumor is 0.01, and
 - a pancreatic islet adenoma tumor is "common" among rats.
- Greater than 50% of the animals in both studies were still alive between weeks 80-90, thus there was adequate exposure of the drug to study tumor incidence.
- Using the log-rank test, the survival rates were not found to be statistically significantly different among the dose groups in either of the two animal studies.

I. Background

Two animal carcinogenicity studies (one in rats, and one in mice) were included in this IND submission. These two studies were intended to assess the oncogenic potential of Montelukast Sodium (MK-0476) in rats and mice when administered orally for two years. The design of these

studies is summarized below.

Study Number	Species	Duration	Doses (mg/kg)
93-110-0	CD-1 (ICR)BR Mouse	92 weeks	0, 0, 25, 50, 200/100*
93-078-0	CD-1 Rat	105 weeks	0, 0, 50, 100, 200

* Due to a treatment-related decrease in body weight gain, the dose level for the high dose group was reduced from 200 to 100 in drug week 10 for both the male and female mice.

In both studies, male and female animals were assigned at random to one of five treatment groups which included two controls and three graded doses of MK-0476 (Mice: 25, 50, 200/100 mg/kg/day; Rats: 50, 100, and 200 mg/kg/day). In the mouse study, due to a treatment-related decrease in body weight gain, the dose level for the high dose group was reduced from 200 to 100 in drug week ten for both the male and female mice. In both studies, the sample size for each sex was 50 for each of two control groups and 50 for each MK-0476 dosage group. The control groups were combined in the analyses to give each study a combined control group size of 100. However, one rat was mis-sexed and excluded from the study in week three resulting in a male rats' combined control group size of 99. Treatment was administered orally (gavage) daily for a period of approximately 92 weeks for the mice and 105 weeks for the rats with terminal necropsy on all remaining animals performed during weeks 92 and 105, respectively, of the mice and rat studies.

Palpable tumors are those which were detected prior to the death or terminal sacrifice of the animal. A nonpalpable tumor was termed "lethal" if classified by the pathologist as a cause of the animal's death (or moribund status leading to an unscheduled sacrifice).

II. Analysis

The sponsor and reviewer analyzed palpable, nonpalpable-lethal and nonpalpable-nonlethal tumors separately, then combined the results using Peto et al. procedures. For a particular tumor type of interest, the incidence data can be summarized in a $2 \times D$ table, where D is the number of dose groups. The first row contains the numbers of animals with the tumor of interest, and the second row contains the numbers of animals without the tumor. However, this summary table can be misleading. If the drug causes animals to die early by some non-cancer related cause, fewer animals will be at risk for tumors in the higher dose groups. Thus, even if the drug also increases the tumor rate, the overall incidence of that tumor in the high dose groups may be smaller than in the control groups. To adjust for the effect that potential differential mortality between the dose groups has on tumor occurrence, the Peto method breaks up study time into several discrete intervals. The intervals used in both studies were: 0-52 weeks, 53-78 weeks, 79-92 weeks, 93-104 weeks, and over 104 weeks. The data can thus be represented by several $2 \times D$ tables, one for each time interval.

The dose groups can also be assigned weights in the statistical analysis to test various hypotheses.

For example, using weights of 0, 1, ... D gives the trend test, which is sensitive to a linear dose effect. Using equal weights (1, 1, 1, 1) gives a test of association between dose and tumor rate without specifying the form of the relationship. Weight can also be made equal to the actual doses given. Finally, choosing weights close to the actual biological effect of the doses will result in the most sensitive test, but in practice this effect is not known. Linear weights or the dose weights are often used.

For the tumor type of interest, each tumor is classified as "fatal", "non-fatal" or "observed before sacrifice or death". This is not a biological classification but a statistical classification. *P*-values are calculated for the three classes separately, and then combined to yield a single *p*-value for the tumor type. Both exact and asymptotic *p*-values can be calculated for tumor type where all of the tumors found were either fatal, non-fatal or observed early. If for a particular tumor type, more than one of the three classes were detected, only asymptotic *p*-values are available. Clearly, when available, the exact *p*-values are preferable.

One-sided *p*-values may be more appropriate than two-sided, since they are more conservative and we are only interested in whether increased doses *increase* tumor incidence.

One hundred forty-one (141) distinct sex/organ/tumor type combinations were found in the two studies. Using an α -level of .05 to determine significance would yield a high false positive rate.

Since so many sex/organ/tumor type combinations are present, a simple application of a .05 decision rule does not appropriately control the overall false positive rate. It has been suggested by Dr. Karl Lin and Dr. Mohammad Rahman¹ that if the tumor is "rare" the cutoff should be .025 and if the tumor is "common" the cutoff should be .005. (Tumors are defined as rare or common using historical control data or the control group in the study being analyzed. The usual practice at FDA is to classify a tumor as common if it occurs in the control group at an incidence of greater than 1%.) Using simulation tests on CD-1 rats and CD(BR) mice, Lin and Rahman found that the overall false positive rate resulting from the use of the α -levels .025 and .005 in the tests for linear trend in a two-species-two-sex study is about 10%. These false-positive rates are judged by the Center for Drug Evaluation and Research as the most appropriate in a regulatory setting.

For pairwise comparisons, the levels of significance are .05 and .01 for a rare and common tumor, respectively.

¹Lin, KK and MA Rahman (1995), "False Positive Rates in Tests for Linear Trends in Tumor Incidences in Animal Carcinogenicity Studies of New Drugs", unpublished report, Division of Biometrics, CDER, FDA, Rockville, MD.

III. Discussion

Dose Weights

As discussed above, it is the usual practice to use either dose weights or linear weights in the analysis of carcinogenicity data. The applicant used dose weights in their analyses. Recall that the mice in the high dose group received 200 mg/kg/day in the first 10 weeks of the study and 100 mg/kg/day after week 10. In the analyses of the mouse study, the applicant selected the 200 mg/kg/day as the highest dose (instead of the 100 mg/kg/day or an intermediate dose). According to the sponsor, the 200 mg/kg/day dose is:

"...the most conservative choice for the male and female mice since it maximizes the differences among the three scales used in the Tukey trend test, and, therefore, will have the greatest chance of obtaining statistical significance..."

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It is assumed that the applicant used the word "conservative" to mean "has the greatest chance of obtaining statistical significance" in the trend tests. Assuming no true tumor trend, the statement is true based on a simulation study conducted by Robert Condon of the Center for Veterinary Medicine at FDA. Additionally, assuming a non-linear tumor trend, the dose weights using the 200 mg dose will also have the greatest chance of obtaining statistical significance. However, assuming a linear tumor trend, the dose weights using the 100 mg dose as the highest dose will have the greatest chance of obtaining statistical significance. Thus, when looking at the Type I error rate, the 200 mg dose is the choice that will have the greatest chance of obtaining statistical significance. However, when looking at power, the most "conservative" choice will depend on the linearity of the true tumor trend.

In the absence of any information about the actual tumor trends for each individual tumor, the p -values in this review reflect a linear dose trend; i.e., the dose groups were given the values (0, 1, 2, 3) in the equations.

Adjusted P -values

As described above, an α -level of .05 is not appropriate because there are 141 unique sex/organ/tumor combinations. Instead of adjusting the α -level at which statistical significance is declared, the applicant adjusted the one-sided p -values using a procedure described by Heyse and Rom² and by Harter³ and then used the usual .05 α -level to determine significance.

Using the adjusted p -values, the applicant found no statistically significant evidence of an

² Heyse, J.F., Rom, D., "Adjusting for Multiplicity of Statistical Tests in the Analysis of Carcinogenicity Studies", *Biometrical Journal* Vol. 30, 1988, 883-896.

³ Harter, H.L., "Error Rates and Sample Sizes for Range Tests in Multiple Comparisons", *Biometrics* Vol. 13, 1957, 511-536.

increasing trend in the incidence of tumor-bearing mice or rats with increasing doses of MK-0476.

Sites In Which Only One Rat Was Observed With Tumor

The applicant's analysis only included sites for which at least two animals were observed with tumor. The applicant argues that statistical significance cannot be achieved for sites in which only one animal was observed with tumor. This is usually true. Since it is possible to find statistical significance, however unlikely, all sites where at least one animal was observed were analyzed in this review.

IV. Reviewer's Analyses and Results

The reviewer's analyses used Peto et al. procedures (described above). The results are on pages 7-9. For both male and female animals, an analysis was performed for each organ/tumor type combination even for cases where only one rat was observed with tumor. The first column in the tables is the sex group, followed by the tumor type and organ. Certain tissue types are labeled as "PRSUNDETER", which indicates that the primary site of the tumor was undetermined. The column labeled "Class" indicates whether the tumors were classified as fatal (FA), non-fatal (NF), observed before sacrifice or death (OB), or mixed (MI), meaning tumors fall into two or more of the former three classes. The incidence in each of the dose groups is shown, although, as discussed above, these may not always be meaningful because the drug may cause the animals to die early by some non-cancer related explanation. Asymptotic and exact p -values are given next, with both one-sided and two-sided p -values shown. (These are denoted by "Asymp1", "Exact1" and "Asymp2" and "Exact2".) Unlike the sponsor, the p -values presented in this review are the actual p -values, not adjusted p -values.

Since the highest dose in the mouse study was reduced from 200 mg/kg/day to 100 mg/kg/day during week 10 of the study, linear dose weights were used in the analyses of this study. To be consistent, linear dose weights were also used in the analyses of the rat study.

As described above, Dr. Karl Lin suggested that if the tumor is "rare" the α -level should be 0.025 and if the tumor is "common" the α -level should be 0.005. Using this rule, there are no statistically significant p -values from the trend test in either of the two animal studies.⁴ This means that as dose increases linearly, there are no statistically significant increases in incidence of tumor. However, the animals in these studies were fed an "optimized diet" which is a modification of a restricted diet regimen; and according to the reviewing pharmacologist Dr. Shannon Williams, a restricted diet can suppress tumor formation. The applicant was asked to send historical control data from studies using this optimized diet and an ad lib diet to help determine which tumors are rare and which are common in this unusual situation. At the time of this review, the data were not available.

⁴ The one-sided exact p -value for the male rats' pancreas islet adenoma tumors is 0.0149. According to the reviewing pharmacologist, this tumor is common, thus the p -value would need to be less than .005 to be considered statistically significant.

The pharmacologist requested pairwise comparisons between each dose level and the control group for five tumor type/organ site combinations in the rat study (page 10). Recall, for pairwise comparisons, the α -levels recommended by Lin are .01 and .05 for common and rare tumors, respectively. The only comparisons that may be statistically significant were the low dose versus control and the high dose versus control for the hepatocellular carcinoma in the liver (50 mg: $p=0.0138$; 200 mg: $p=0.0394$). However in this study, the control group's incidence was 2.02%. Recall that the usual practice at FDA is to classify a tumor as common if it occurs in the control group at an incidence of greater than 1%. Thus, the pharmacologist may want to study the historical control data to be submitted by the applicant to decide whether this p -value is statistically significant or not. The p -values of the middle and high dose group comparisons with placebo for Pancreatic Islet Adenoma tumors were .0301 and .0397 respectively. Pancreatic islet adenoma tumors are common, thus the p -values were not statistically significant. All of the other pairwise comparisons requested by Dr. Williams yielded p -values greater than .05.

The pharmacologist considered combining types of tumors within tissue type based on McConnell et al (1986)⁵. However, from inspection after grouping the tumors, it was apparent that there were no increasing tumor trends.

Survival

In the Guidance for Industry draft, it is stated that "a 50% survival rate of the 50 initial animals in the high dose group between weeks 80-90 of a two-year study will be considered as a sufficient number and adequate exposure."⁶ For both the mouse and rat study, plots of survival demonstrate that greater than 50% of the high dose group animals were still alive during weeks 80-90 (page 11).

As discussed above, the trend test used in the applicant's and reviewer's analyses take into account any potential difference in survival rates. Nevertheless, Kaplan-Meier plots and log-rank tests were used to determine if the survival rates among the different dose groups were similar (page 11). Neither the plots nor the log-rank test p -values show any statistically significant evidence of a difference in survival among the dose groups.

⁵ McConnell, EE, HA Solleveld, JA Swenberg and GA Boorman. "Guidelines for Combining Neoplasms for Evaluation of Rodent Carcinogenesis Studies", *Journal of National Cancer Institute* 1986; 76:283-289.

⁶ Guidance For Industry, "On Statistical Aspects of Design, Analysis, and Interpretation of Animal Carcinogenicity Studies."

P-values from the Trend Test

Mouse Study

Sex Tumor Type	Tissue	Class	C	L	M	H	Asymp2	Exact2	Asymp1	Exact1
F ADENOMA	LUNG	NF	8	9	2	11	0.1116	0.1149	0.0558	0.0671
M HEMANGIOMA	TESTIS	NF	0	0	0	1	0.1204	0.2030	0.0602	0.2030
M HEMANGIOMA	SPLEEN	NF	0	0	0	1	0.1204	0.2030	0.0602	0.2030
M HEMANGIOSARCOMA	LYMPHNODE	NF	0	0	0	1	0.1204	0.2030	0.0602	0.2030
F FIBROSARCOMA	SKIN	OB	0	2	1	2	0.1216	0.1302	0.0608	0.0928
M PAPILLOMA	SKIN	OB	0	0	0	1	0.1227	0.2000	0.0614	0.2000
F ADENOMA	ADRENACORT	NF	0	0	0	1	0.1248	0.2018	0.0624	0.2018
F LYMPHOMA	PRSUDETER	MI	9	3	7	8	0.1628	NA	0.0814	NA
M ADENOMA	LUNG	NF	17	12	10	12	0.2633	0.2735	0.1317	0.1473
M ADENOMA	THYRFOLLIC	NF	0	2	0	1	0.3026	0.4445	0.1513	0.2313
M ADENOMA	PITUITARY	NF	0	0	1	0	0.3156	0.3438	0.1578	0.3438
M LIPOSARCOMA	LIVER	NF	0	0	1	0	0.3156	0.3438	0.1578	0.3438
F ADENOCARCINOMA	SMAINTESTI	NF	0	1	0	1	0.3341	0.4018	0.1671	0.2400
M ADENOMA	PANCREAISL	NF	0	0	1	0	0.4864	0.7871	0.2432	0.3861
M ADENOMA	PROSTATE	NF	0	0	1	0	0.4864	0.7871	0.2432	0.3861
M HEMANGIOMA	PERITONEUM	NF	0	0	1	0	0.4864	0.7871	0.2432	0.3861
M SERTOLICELLTUMOR	TESTIS	NF	0	0	1	0	0.4864	0.7871	0.2432	0.3861
M SQUAMOUSCELLCARCINOMA	EAR	NF	0	0	1	0	0.4864	0.7871	0.2432	0.3861
F HISTIOCYTOMA	SKIN	NF	0	0	1	0	0.4956	0.8073	0.2478	0.4037
F LEIOMYOSARCOMA	SMAINTESTI	NF	0	0	1	0	0.4956	0.8073	0.2478	0.4037
F ADENOMA	PITUITARY	NF	1	2	2	1	0.5282	0.6050	0.2641	0.3221
M ADENOMA	ADRENACORT	NF	5	3	2	4	0.5345	0.5568	0.2673	0.3050
F ADENOMA	OVARY	NF	2	1	0	2	0.7026	0.8502	0.3513	0.4163
M SPINDLECELLTUMOR	ADRENAL	NF	1	0	0	1	0.7046	0.8079	0.3523	0.4767
M HEMANGIOSARCOMA	SKELETMUSC	OB	0	1	1	0	0.7057	0.8038	0.3528	0.4756
F GRANULOSACELLTUMO	OVARY	NF	2	1	2	1	0.7809	0.8644	0.3904	0.4515
F SPINDLECELLTUMOR	ADRENAL	NF	1	2	0	1	0.9340	1.0000	0.4670	0.4601
F ADENOCARCINOMA	MAMMARGLAN	MI	3	5	1	2	0.9588	NA	0.5206	NA
M LYMPHOMA	PRSUDETER	MI	2	3	1	1	0.9484	NA	0.5258	NA
F POLYP	UTERUS	NF	1	3	2	0	0.9407	1.0000	0.5297	0.5485
F HISTIOCYTICSARCOM	PRSUDETER	MI	1	3	1	0	0.9390	NA	0.5305	NA
M POLYP	LARINTESTI	NF	1	0	1	0	0.9364	1.0000	0.5318	0.5947
M ADENOMA	SMAINTESTI	NF	0	1	0	0	0.8719	1.0000	0.5641	0.6139
M NEUROFIBROMA	PLEURA	NF	0	1	0	0	0.8719	1.0000	0.5641	0.6139
M HISTIOCYTICSARCOM	PRSUDETER	FA	0	1	0	0	0.8638	1.0000	0.5681	0.6000
F ADENOCARCINOMA	EHARDERIGL	NF	0	1	0	0	0.8632	1.0000	0.5684	0.5963
F ADENOMA	UTERUS	NF	0	1	0	0	0.8632	1.0000	0.5684	0.5963
F HEMANGIOSARCOMA	UTERUS	NF	0	1	0	0	0.8632	1.0000	0.5684	0.5963
F LEIOMYOSARCOMA	UTERUS	NF	0	1	0	0	0.8632	1.0000	0.5684	0.5963
M HEMANGIOSARCOMA	LIVER	MI	1	1	1	0	0.7661	NA	0.6169	NA
F ADENOMA	THYRFOLLIC	NF	1	1	1	0	0.7643	0.8334	0.6178	0.5034
F HEPATOCELLULARADENOMA	LIVER	NF	4	3	3	1	0.7478	0.7962	0.6261	0.5678
F ADENOCARCINOMA	LUNG	MI	6	3	5	1	0.6215	NA	0.6892	NA
F ADENOMA	EHARDERIGL	NF	8	2	3	3	0.5945	0.6626	0.7028	0.6583
M PHEOCHROMOCYTOMA	ADRENAL	NF	1	1	0	0	0.4030	0.5659	0.7985	0.6688
F SARCOMA	UTENDOMETS	MI	3	0	2	0	0.3985	NA	0.8008	NA
M HEMANGIOSARCOMA	SPLEEN	MI	1	1	0	0	0.3959	NA	0.8021	NA
M ADENOMA	EHARDERIGL	NF	11	7	4	3	0.3656	0.4131	0.8172	0.7898
M ADENOCARCINOMA	LUNG	MI	14	1	5	4	0.3359	NA	0.8321	NA
M ADENOCARCINOMA	EHARDERIGL	NF	1	0	0	0	0.3084	0.6040	0.8458	0.5990
M HEMANGIOMA	LYMPHNODE	NF	1	0	0	0	0.3084	0.6040	0.8458	0.5990
M OSTEOMA	BONE	NF	1	0	0	0	0.3084	0.6040	0.8458	0.5990
M POLYP	GALLBLADDE	NF	1	0	0	0	0.3084	0.6040	0.8458	0.5990
F ADENOCARCINOMA	UTERUS	NF	1	0	0	0	0.3049	0.6055	0.8476	0.5963
F ADENOMA	SMAINTESTI	NF	1	0	0	0	0.3049	0.6055	0.8476	0.5963
F HEMANGIOMA	UTERUS	NF	1	0	0	0	0.3049	0.6055	0.8476	0.5963
F HEMANGIOMA	SKIN	NF	1	0	0	0	0.3049	0.6055	0.8476	0.5963
F HEMANGIOMA	SPLEEN	NF	1	0	0	0	0.3049	0.6055	0.8476	0.5963
F HEMANGIOSARCOMA	LIVER	NF	1	0	0	0	0.3049	0.6055	0.8476	0.5963
F LEIOMYOSARCOMA	OVARY	NF	1	0	0	0	0.3049	0.6055	0.8476	0.5963

F MENINGIOMA	BRAIN	NF	1	0	0	0	0.3049	0.6055	0.8476	0.5963
F OSTEOOMA	BONE	NF	1	0	0	0	0.3049	0.6055	0.8476	0.5963
F OSTEOSARCOMA	PRSUDETER	NF	1	0	0	0	0.3049	0.6055	0.8476	0.5963
F SEBACEOUSADENOMA	SKIN	NF	1	0	0	0	0.3049	0.6055	0.8476	0.5963
F TERATOMA	OVARY	NF	1	0	0	0	0.3049	0.6055	0.8476	0.5963
F BASALCELLTUMOR	SKIN	OB	1	0	0	0	0.3035	0.6000	0.8483	0.6000
M TRICHOEPITHELIOMA	SKIN	OB	1	0	0	0	0.3035	0.6000	0.8483	0.6000
M HEPATOCELLULARCARCINOMA	LIVER	MI	17	5	5	5	0.2523	NA	0.8738	NA
M HEPATOCELLULARADENOMA	LIVER	NF	14	8	7	3	0.2474	0.2516	0.8763	0.8584
F HEPATOCELLULARCARCINOMA	LIVER	NF	3	1	1	0	0.2462	0.2688	0.8769	0.8255
M FIBROSARCOMA	SKIN	MI	3	1	1	0	0.2184	NA	0.8908	NA
M ADENOCARCINOMA	SMAINTESTI	MI	2	1	0	0	0.1974	NA	0.9013	NA
M LIPOMA	SKIN	OB	2	0	0	0	0.1501	0.2786	0.9250	0.8358
M ADENOMA	TESTLEYDCE	NF	2	0	0	0	0.1487	0.2747	0.9256	0.8404
M LEUKEMIA	PRSUDETER	NF	2	0	0	0	0.1487	0.2747	0.9256	0.8404
M POLYP	URINABLADD	NF	2	0	0	0	0.1487	0.2747	0.9256	0.8404
F POLYP	GALLBLADDE	NF	2	0	0	0	0.1458	0.2837	0.9271	0.8382
F ADENOACANTHOMA	MAMMARGLAN	MI	3	1	0	0	0.1037	NA	0.9481	NA
F LEIOMYOMA	UTERUS	NF	6	1	2	0	0.0917	0.1111	0.9542	0.9417
M HEMANGIOMA	LIVER	NF	4	0	0	0	0.0401	0.0470	0.9799	0.9753

Rat Study

Sex Tumor Type	Tissue Class	C	L	M	H	Asymp2	Exact2	Asymp1	Exact1	
M ADENOMA	PANCREAISL	NF	3	4	6	6	0.0212	0.0239	0.0106	0.0149
F PAPILOMA	STNONGLANM	NF	0	0	0	2	0.0364	0.0498	0.0182	0.0498
F ADENOCARCINOMA	UTERUS	MI	0	1	1	2	0.0811	NA	0.0406	NA
F ADENOMA	KIDNEY	NF	1	0	0	3	0.0897	0.1402	0.0448	0.0755
M HEPATOCELLULARCARCINOMA	LIVER	MI	2	6	3	5	0.0902	NA	0.0451	NA
M MESOTHELIOMA	HEART	FA	0	0	0	1	0.1237	0.2008	0.0618	0.2008
M FIBROADENOMA	MAMMARGLAN	OB	0	0	0	1	0.1237	0.2008	0.0618	0.2008
F GLIOMA	BRAIN	NF	0	0	0	1	0.1247	0.1923	0.0623	0.1923
M ADENOCARCINOMA	LAINTESTCO	NF	0	0	0	1	0.1256	0.1964	0.0628	0.1964
M ADENOMA	MAMMARGLAN	NF	0	0	0	1	0.1256	0.1964	0.0628	0.1964
M HEMANGIOMA	SKELETMUSC	NF	0	0	0	1	0.1256	0.1964	0.0628	0.1964
M PAPILOMA	TONGUE	NF	0	0	0	1	0.1256	0.1964	0.0628	0.1964
F MESOTHELIOMA	PERITONEUM	NF	0	0	0	1	0.1402	0.2256	0.0701	0.2256
F SQUAMOUSCELLCARCINOMA	SKIN	NF	0	0	0	1	0.1402	0.2256	0.0701	0.2256
M KERATOACANTHOMA	SKIN	OB	0	2	3	1	0.1779	0.2242	0.0890	0.1256
M HISTIOCYTICSARCOM	PRSUDETER	MI	1	0	0	2	0.2354	NA	0.1177	NA
F POLYP	UTERUS	NF	5	4	8	4	0.2834	0.2867	0.1417	0.1656
F ADENOMA	PANCREAISL	NF	1	1	1	2	0.2884	0.3523	0.1442	0.1962
M GLIOMA	BRAIN	NF	0	0	2	0	0.3073	0.4011	0.1536	0.2265
F ADENOMA	THYRFOLLIC	NF	0	0	2	0	0.3721	0.5661	0.1860	0.2623
F HISTIOCYTICSARCOM	PRSUDETER	OB	0	0	1	0	0.4927	0.8000	0.2464	0.4000
M ADENOCARCINOMA	MAMMARGLAN	OB	0	0	1	0	0.4953	0.7992	0.2476	0.4016
M ADENOMA	PANCREACIN	NF	0	0	1	0	0.5022	0.8095	0.2511	0.4167
M HEMANGIOMA	LYMPHNODE	NF	0	0	1	0	0.5022	0.8095	0.2511	0.4167
M THYMOMA	THYMUS	NF	0	0	1	0	0.5022	0.8095	0.2511	0.4167
F ADENOCARCINOMA	PANCREAISL	NF	0	0	1	0	0.5292	0.7866	0.2646	0.4085
F ADENOMA	PARATHYROI	NF	0	0	1	0	0.5292	0.7866	0.2646	0.4085
F ADENOMA	LIVEBILDUC	NF	0	2	0	1	0.5450	0.6305	0.2725	0.3566
M INTERSTITIALCELLTUMOR	TESTIS	NF	2	4	2	2	0.6150	0.6789	0.3075	0.3552
M HEPATOCELLULARADENOMA	LIVER	NF	3	5	4	2	0.6336	0.6398	0.3168	0.3570
M ADENOMA	SKSEBACEGL	OB	0	1	1	0	0.7175	0.8000	0.3587	0.4808
M PAPILOMA	SKIN	OB	1	0	0	1	0.7214	0.8000	0.3607	0.4828
M PAPILOMA	MOUTHLP	OB	0	1	1	0	0.7238	0.7971	0.3619	0.4833
F FIBROMA	SKIN	MI	1	1	0	1	0.7306	NA	0.3653	NA
M GRANULARCELLTUMOR	BRAIN	NF	1	0	0	1	0.7327	0.7906	0.3664	0.4871
F MELANOMA	EYEIRIS	NF	0	1	1	0	0.7698	1.0000	0.3849	0.4875
M FIBROSARCOMA	SKIN	OB	1	0	1	0	0.7949	1.0000	0.3975	0.4878
F FIBROSARCOMA	SKIN	OB	1	0	1	0	0.8109	1.0000	0.4054	0.4785
F FIBROADENOMA	MAMMARGLAN	MI	30	14	19	14	0.8674	NA	0.4337	NA
F PHEOCHROMOCYTOMA	ADRENMEDUL	NF	1	1	0	1	0.9094	1.0000	0.4547	0.4700
M LIPOSARCOMA	KIDNEY	MI	2	0	1	1	0.9656	NA	0.4828	NA

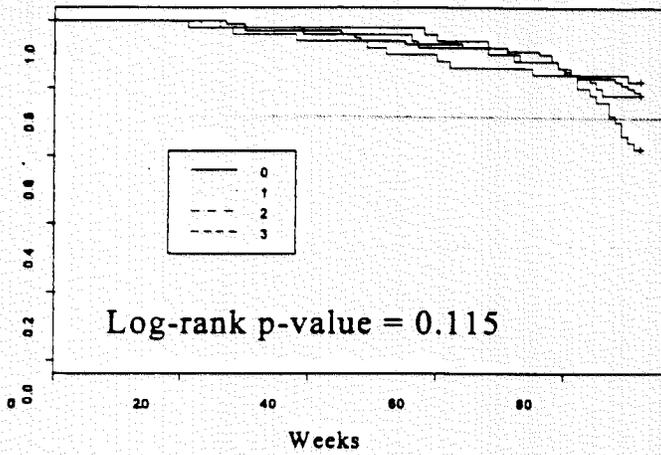
F LYMPHOMA	PRSUNDETER	NF	1	0	1	0	0.9825	1.0000	0.4913	0.3902
M ADENOMA	PARATHYROI	NF	0	1	0	0	0.9194	1.0000	0.5403	0.6667
M HISTIOCYTICSARCOM	PROSTATE	FA	0	1	0	0	0.8606	1.0000	0.5697	0.5984
M MELANOMA	EYE	FA	0	1	0	0	0.8606	1.0000	0.5697	0.5984
M HEMANGIOSARCOMA	SKIN	OB	0	1	0	0	0.8606	1.0000	0.5697	0.5984
M SQUAMOUSCELLCARCINOMA	EAEXTERNAE	OB	0	1	0	0	0.8606	1.0000	0.5697	0.5984
M TRICHOEPITHELIOMA	SKIN	OB	0	1	0	0	0.8606	1.0000	0.5697	0.5984
M ACINAR-ISLETCELLTUMOR	PANCREAS	NF	0	1	0	0	0.8497	1.0000	0.5752	0.5833
M ADENOCARCINOMA	LUNG	NF	0	1	0	0	0.8497	1.0000	0.5752	0.5833
M ADENOMA	LUNG	NF	0	1	0	0	0.8497	1.0000	0.5752	0.5833
M HEMANGIOSARCOMA	SPLEEN	NF	0	1	0	0	0.8497	1.0000	0.5752	0.5833
F ADENOMA	VAGCLITOGL	NF	0	1	0	0	0.8285	1.0000	0.5857	0.5915
M ADENOMA	THYRFOLLIC	NF	1	0	1	0	0.7881	1.0000	0.6060	0.5129
M MESOTHELIOMA	TESTUVAGIN	NF	1	1	1	0	0.7843	0.8205	0.6079	0.5129
F LEIOMYOSARCOMA	UTERUS	MI	0	2	0	0	0.7633	NA	0.6183	NA
M ADENOMA	ADRENACORT	NF	3	0	1	1	0.6679	0.7117	0.6660	0.5815
F ADENOCARCINOMA	PITUITARY	MI	3	1	1	1	0.6017	NA	0.6991	NA
F ADENOCARCINOMA	MAMMARGLAN	MI	19	7	2	10	0.4827	NA	0.7586	NA
M LIPOMA	SKIN	MI	1	2	0	0	0.4271	NA	0.7864	NA
M CARCINOMA	TPARAFOLL	NF	4	1	1	1	0.4221	0.5183	0.7890	0.7297
M HISTIOCYTOMA	SKIN	MI	2	1	1	0	0.3983	NA	0.8009	NA
M ADENOCARCINOMA	PANCREAISL	MI	7	2	2	2	0.3848	NA	0.8076	NA
F ADENOMA	MAMMARGLAN	MI	3	2	0	1	0.3844	NA	0.8078	NA
M PHEOCHROMOCYTOMA	ADRENMEDUL	NF	8	3	3	2	0.3595	0.3820	0.8202	0.7867
M ADENOMA	EZYMBALGLA	NF	1	0	0	0	0.3337	0.6111	0.8332	0.5556
M TRANSITIONALCELLCARCINOMA	URINABLADD	NF	1	0	0	0	0.3337	0.6111	0.8332	0.5556
F GRANULARCELLTUMOR	BRAIN	NF	1	0	0	0	0.3112	0.6154	0.8444	0.5769
F HISTIOCYTICSARCOM	LIVER	FA	1	0	0	0	0.3035	0.6000	0.8483	0.6000
F LEIOMYOSARCOMA	LARINTESTA	FA	1	0	0	0	0.3035	0.6000	0.8483	0.6000
F FIBROSARCOMA	EYELID	OB	1	0	0	0	0.3035	0.6000	0.8483	0.6000
F KERATOACANTHOMA	SKIN	OB	1	0	0	0	0.3035	0.6000	0.8483	0.6000
F PAPILOMA	MOUTHLIP	OB	1	0	0	0	0.3035	0.6000	0.8483	0.6000
M BASALCELLTUMOR	SKIN	OB	1	0	0	0	0.3015	0.5984	0.8493	0.6024
M FIBROSARCOMA	EARPINNA	OB	1	0	0	0	0.3015	0.5984	0.8493	0.6024
M ADENOMA	KIDNEY	NF	1	0	0	0	0.2937	0.5893	0.8532	0.6071
F ADENOCARCINOMA	KIDNEY	NF	1	0	0	0	0.2880	0.6037	0.8560	0.6220
F CARCINOMA	STNONGLANM	NF	1	0	0	0	0.2880	0.6037	0.8560	0.6220
F FIBROSARCOMA	EARPINNA	NF	1	0	0	0	0.2880	0.6037	0.8560	0.6220
F PAPILOMA	SKIN	NF	1	0	0	0	0.2880	0.6037	0.8560	0.6220
M FIBROMA	SKIN	MI	8	2	0	3	0.2723	NA	0.8638	NA
M PAPILOMA	URINABLADD	NF	2	1	0	0	0.2125	0.3066	0.8937	0.8182
M LYMPHOMA	PRSUNDETER	MI	5	3	0	1	0.1968	NA	0.9016	NA
F GRANULOSACELLTUMOR	OVARY	NF	2	0	0	0	0.1879	0.2821	0.9061	0.7900
F CARCINOSARCOMA	MAMMARGLAN	OB	2	0	0	0	0.1468	0.2786	0.9266	0.8393
M ADENOMA	PITUITARY	MI	40	15	19	13	0.1420	NA	0.9290	NA
F CARCINOMA	TPARAFOLL	NF	2	0	0	0	0.1318	0.1913	0.9341	0.8585
F HEPATOCELLULARADENOMA	LIVER	NF	7	3	2	1	0.1289	0.1475	0.9355	0.9202
F ADENOMA	ADRENACORT	NF	4	4	0	0	0.1137	0.1204	0.9432	0.9258
F ADENOMA	PITUITARY	MI	69	30	33	27	0.0696	NA	0.9652	NA
M ADENOMA	TPARAFOLL	NF	14	4	2	3	0.0459	0.0473	0.9771	0.9737
F ADENOMA	TPARAFOLL	NF	13	3	3	2	0.0282	0.0309	0.9859	0.9840

Pairwise Comparisons of Neoplastic Findings in Rats

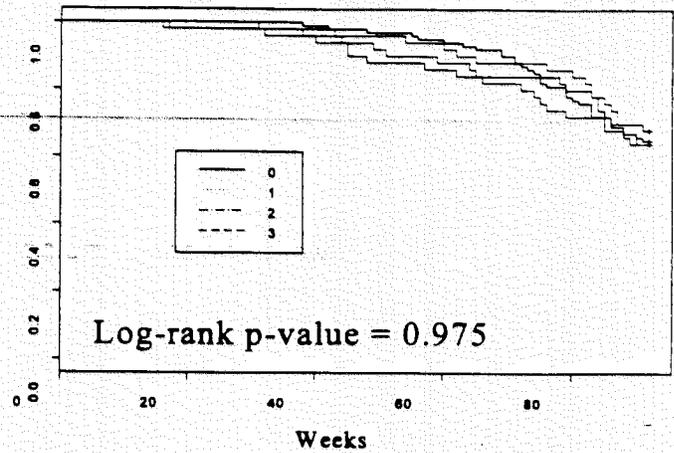
Male Rats				
	Controls 1+2	50 mg	100 mg	200 mg
Total number of animals	99	50	50	50
Number of animals with tumor (<i>p</i> -value of pairwise comparison with control groups)				
<i>Liver</i> : Hepatocellular carcinoma	2	6 (0.0138)	3 (0.2171)	5 (0.0394)
<i>Pancreas</i> : Islet adenoma	3	4 (0.1471)	6 (0.0301)	6 (0.0397)
<i>Brain</i> : Malignant glioma	0	0	2 (0.0958)	0
Female Rats				
	Controls 1+2	50 mg	100 mg	200 mg
Total number of animals	100	50	50	50
Number of animals with tumor (<i>p</i> -value of pairwise comparison with control groups)				
<i>Stomach</i> : Non-glandular mucosa papilloma	0	0	0	2 (0.1401)
<i>Uterus</i> : Adenocarcinoma	0	1 (0.2667)	1 (0.3366)	2
<i>Pancreas</i> : Islet adenoma	1	1 (0.5340)	1 (0.5663)	2 (0.2885)
<i>Brain</i> : Malignant glioma	0	0	0	1 (0.3125)

Kaplan-Meier Plots

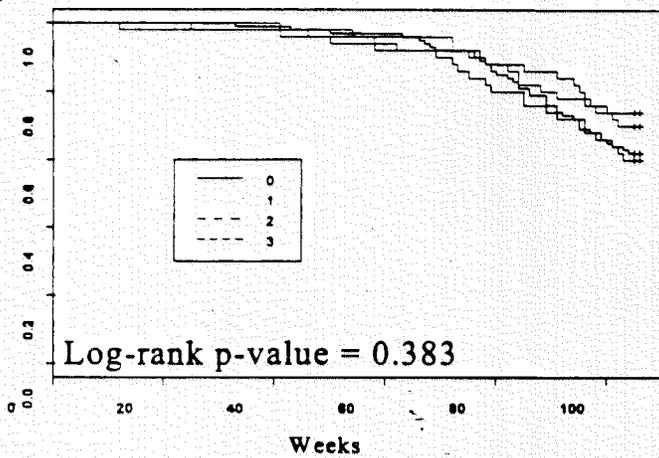
Female Mice



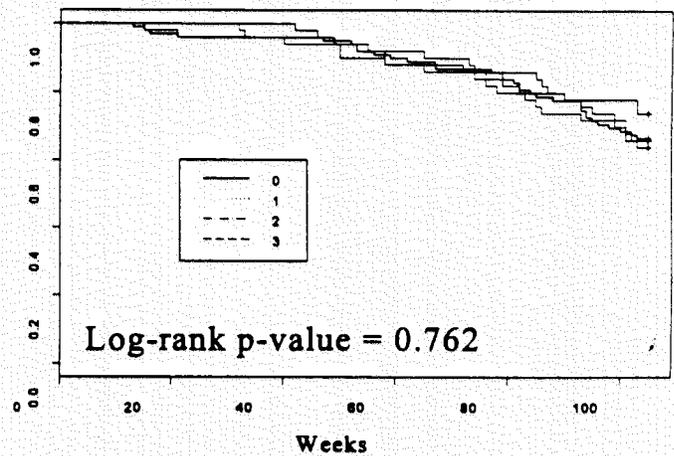
Male Mice



Female Rats



Male Rats



ISI 8/12/96

Barbara A. Bono

concur: Dr. Lin
Dr. Nevius ISI 8/12/96
8-12-96

cc:

Orig.

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