CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-908

STATISTICAL REVIEW(S)

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

STATISTICAL REVIEW AND EVALUATION

CLINICAL STUDIES

NDA/Serial Number:	21-908
Drug Name:	RU-0211 (lubiprostone) tablet
Indication(s):	—
Applicant:	Sucampo Pharmaceuticals, Inc.
Date(s):	eCTD dated March 16, 2005
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Keywords: clinical study, placebo-controlled, LOCF

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1. EXECUTIVE SUMMARY

1.1 Conclusions and Recommendations

The sponsor has submitted two pivotal studies (SC0131 and SC0232) to support the claim.

Study SC0131 showed that RU-0211 48 μg group was statistically significantly better than placebo group in terms of the primary efficacy endpoint, the spontaneous bowel movements (SBMs) frequency rate during Week1 in subjects with constipation. The superiority was also shown for all secondary efficacy endpoints with exceptions for abdominal bloating and abdominal discomfort.

The efficacy results from study SC0131 were replicated in study SC0232 for the primary efficacy endpoint and for the most of secondary efficacy variables (SBM within 24 hours of first study drug, time to first SBM, degree of stool consistency, degree of straining, degree of constipation and global assessment of treatment effectiveness, abdominal bloating and abdominal discomfort).

For study SC0232, the sponsor's ITT analysis was not true ITT analysis. It did not include all randomized patients. It excluded more patients in RU-0211 48 μg group than in placebo group (8 vs. 1, p=0.0180, chi-square test). So, sponsor's ITT analysis may tend to be biased in favor of RU-0211 48 μg group.

Furthermore, in the sponsor's analysis of weekly response rate, it was also found that more patients in RU-0211 48 μg group were imputed by LOCF than in placebo at Weeks 3 and 4 (8 in RU-0211 48 μg group and 2 in placebo group). So, the sponsor's ITT analysis for weekly response rate may tend to be biased in favor of RU-0211 48 μg group.

As re-analysis for weekly response rate, this reviewer performed CMH (Cochran-Mantel-Haenszel) test using modified ridit scores for reviewer's ITT population without LOCF. In these analyses, patients with missing outcomes were set as no responders. Contrary to the sponsor's finding based on sponsor's ITT analysis with LOCF, it was found that treatment difference achieved statistical significance only at Week 1 (primary efficacy assessment time point) and Week 3 at significance level of 0.05 without adjustment for multiplicity.

For the more clinically meaningful efficacy parameter, where a responder is defined as a patient who had an SBM frequency rate of ≥ 3 per week for all 4 weeks, and who did not use rescue medication during or within 24 hours prior to the given week, and who did not drop out during the study due to lack of efficacy, this reviewer performed the responder analysis. In this analysis, patients with missing outcomes were set as no responders. This endpoint is more stringent than the pre-specified primary endpoint.

Both studies (SC0131 and SC0232) showed that for more stringent efficacy endpoint (≥ 3 SBMs/wk for all 4 weeks), the RU-0211 48 μg was superior to the placebo with treatment differences of about 20% and 16% for studies SC0131 and SC0232, respectively.

In Study SC9921, a dose ranging Phase IIb study, the primary efficacy endpoint was pre-specified as number of SBMs. The primary time point was pre-specified as Week 3. There was slight imbalance in weekly average number of SBMs at baseline (1.1 for 24 μg, 1.3 to 1.4 for other groups). It failed to achieve statistical significance due to inadequate sample size. So, the efficacy analysis based on weekly average of SBM might be biased in favor of higher doses. It is more appropriate to asses the efficacy results based on the change from baseline.

This reviewer performed analysis of change of weekly average number of SBMs from baseline using Wilcoxon test. Results of this analysis showed that all doses were statistically significant from placebo at week 1 and week 2. But, they failed to achieve statistical significance at week 3. No differences between the low dose (24 μg) and middle dose (48 μg) were observed at week 2 and week 3. At week 1, middle dose (48 μg) was numerically slightly better than low dose (24 μg).

This reviewer performed post-hoc analyses for two stringent efficacy endpoints, the number of patients with increase of greater than or equal one SBM/wk from baseline and number of patients with greater than or equal to 3 SBMs/wk. These efficacy endpoints were used for approval of Zelnorm. In the Zelnorm submission, complete spontaneous bowel movement (CSBM)/wk was assessed instead of SBMs/wk.

The results of this reviewer's analyses for the number of patients with increase greater than or equal to one SBM/week and number of patients with greater than or equal to 3 SBMs/wk showed that all doses were numerically better than placebo for both two stringent endpoints and for all timepoints (week 1, week 2, week 3, and over the period week 1 to week 3). But, they failed to achieve statistical significance due to insufficient sample sizes. Furthermore, the low dose (24 μg) was close to the middle dose (48 μg) at week 1, week 3, and week 1 to week 3. However, the low dose was slightly better than the middle dose at week 2. So, the minimum effective dose might be the low dose ((24 μg). The low dose (24 μg) should be included in the Phase III studies.

In conclusion, both studies (SC0131 and SC0232) showed that the RU-0211 48 μg was superior to the placebo for pre-specified primary efficacy endpoint and most secondary efficacy endpoints. Even for more stringent efficacy endpoint (≥ 3 SBMs/wk for all 4 weeks), the reviewer's post-hoc analysis revealed that the RU-0211 48 μg was superior to the placebo with treatment differences of about 20% and 16% for studies SC0131 and SC0232, respectively. However, the results form reviewer's post hoc analysis for Study SC9921 revealed that the RU-0211 48 μg might not be the minimum effective dose.

1.2 Brief Overview of Clinical Studies

The sponsor has submitted three adequate and well-controlled studies (SC9921, SC0131 and SC0232) to support the claim. Studies SC0131 and SC0232 were carried out using identical study design and these trials are considered the pivotal studies for this submission. Study SC9921 was a Phase IIb dose-ranging study that utilized a similar study design.

1.2.1 Study SC0232

This study was a double-blind, randomized, multi-site (20 sites), placebo controlled study for the treatment of occasional constipation.

The objective of this study was to evaluate the efficacy and safety of 48 µg RU-0211 (24 µg RU-0211 b.i.d.) when compared with placebo for the treatment of occasional constipation.

Subjects with a documented history of constipation, defined as, on average, < 3 SBMs per week as confirmed during the baseline/washout period and who were symptomatic for constipation were eligible for enrollment in the study.

This study consisted of a two week baseline/washout period, followed by a four week, randomized, double-blind, placebo-controlled treatment period and a follow-up visit 14 days after the end of treatment.

No rescue medications were allowed during Week 1 of treatment; however, the study design did allow for administration of rescue medication during both the baseline/washout period and during Treatment Weeks 2, 3, and 4 of treatment, but only as prescribed by the investigator. After 3 consecutive days of not having a spontaneous BM, the investigator might prescribe to the subject a 10 mg bisacodyl suppository. If this was not effective, a Fleet enema would be used. No rescue medication would be allowed in the 48 hours before randomization into the double-blind period.

Following initial assessment and after completing a 2-week baseline/washout, eligible subjects were randomly assigned to receive either RU-0211 24µg oral capsules twice daily (b.i.d.) or matching placebo b.i.d. for 4 weeks.

All subjects were to take 1 capsule (RU-0211 24 µg or placebo) 2 times each day (AM and PM doses, at breakfast and dinner with at least 8 ounces of water).

The study consisted of a screening visit (Visit 1, Days -15 to -2), an enrollment visit (Visit 2, Day -1), 2 interim visits (Visit 3, Day 8 and Visit 4, Day 15; these occurred after 1 and 2 weeks of treatment), an end of treatment visit (Visit 5, Day 29), and follow-up evaluation (Visit 6, Day 43) approximately 2 weeks after Visit 5.

Subjects were considered evaluable for efficacy if they were randomized and took at least 1 dose of double-blind study medication.

The "last observation carried forward" (LOCF) method would be used to impute missing values primary caused by early withdrawal from the study.

The primary efficacy variable was the frequency of SBMs at Week 1. An SBM was defined as any BM that did not occur within 24 hours after rescue medication use.

The secondary efficacy variables were as follows:

- Frequency of SBMs at Weeks 2, 3, and 4 and all bowel movements (BMs) at Weeks 1, 2, 3, and 4.
- Weekly responder rates
- Percentage of subjects with an SBM within 24 hours of first study drug administration
- Time to first SBM
- Weekly symptomatic assessment of average degree of stool consistency, average degree of straining, average degree of severity of constipation, global assessment of treatment effectiveness, and abdominal symptoms (bloating and discomfort).

1.2.2 Study SC0131

The study design for this study was similar to those for Study SC0132 with few exceptions listed below.

The secondary efficacy variables did not include all bowel movements (BMs) at Weeks 1, 2, 3, and 4.

1.2.3 Study SC9921

This study was a double-blind, randomized, multi-center, placebo controlled study for the treatment of occasional constipation.

The objective of this study was to assess the efficacy and safety of different dose regimens of oral RU-0211 (24 μg, 48 μg, and 74 μg) compared to placebo on relief of chronic constipation as assessed by the number of spontaneous bowel movements and abdominal symptoms.

Chronic constipation was identified as < 3 SBMs per week, on average, accompanied by at least 1 symptom of constipation for at least 6 months.

The duration of treatment was 21 days.

The primary efficacy endpoint was daily average number of SBMs.

The secondary efficacy endpoints were:
1). Percentage of patients with a SBMs on Day1

2). Average degree of evacuation
3). Average degree of straining
4). Average stool consistency
5). Assessments of abdominal bloating and discomfort
6). Global assessment of constipation
7). Global assessment of treatment effectiveness
8). Usage of rescue medication
9). Percentage of patients using the rescue medication
10). Percentage of treatment failure

All randomized subjects who tool at least one dose of study drug constituted the population of the "intent-to-treat" population.

For all inferential analyses of efficacy, the between-group comparisons were performed between the placebo group and each of the RU-0211 groups.

1.3 Statistical Issues and Findings

The sponsor has submitted two pivotal studies (SC0131 and SC0232) to support the claim.

For study SC0232, the sponsor's ITT analysis was not true ITT analysis. It did not include all randomized patients. It excluded more patients in RU-0211 48 μg group than in placebo group (8 vs. 1, p=0.0180, chi-square test). So, sponsor's ITT analysis may tend to be biased in favor of RU-0211 48 μg group.

Furthermore, in the sponsor's analysis of weekly response rate, it was also found that more patients in RU-0211 48 μg group were imputed by LOCF than in placebo at Weeks 3 and 4 (8 in RU-0211 48 μg group and 2 in placebo group). So, the sponsor's ITT analysis for weekly response rate may tend to be biased in favor of RU-0211 48 μg group.

As re-analysis for weekly response rate, this reviewer performed CMH (Cochran-Mantel-Haenszel) test using modified ridit scores for reviewer's ITT population without LOCF. In these analyses, patients with missing outcomes were set as no responders. Contrary to the sponsor's finding based on sponsor's ITT analysis with LOCF, it was found that treatment difference achieved statistical significance only at Week 1 (primary efficacy assessment time point) and Week 3 at significance level of 0.05 without adjustment for multiplicity.

For the more clinically meaningful efficacy parameter, where a responder is defined as a patient who had an SBM frequency rate of ≥ 3 per week for all 4 weeks, and who did not use rescue medication during or within 24 hours prior to the given week, and who did not drop out during the study due to lack of efficacy, this reviewer performed the responder analysis. In this analysis, patients with missing outcomes were set as no responders. This endpoint is more stringent than the pre-specified primary endpoint.

Both studies (SC0131 and SC0232) showed that for more stringent efficacy endpoint (≥ 3 SBMs/wk for all 4 weeks), the RU-0211 48 μg was superior to the placebo with treatment

differences of about 20% (41% for 48μg and 21% for placebo) and 16% (44% for 48μg and 28% for placebo) for studies SC0131 and SC0232, respectively.

In Study SC9921, a dose ranging Phase IIb study, the primary efficacy endpoint was pre-specified as number of SBMs. The primary time point was pre-specified as Week 3. There was slight imbalance in weekly average number of SBMs at baseline (1.1 for 24 μg, 1.3 to 1.4 for other groups). It failed to achieve statistical significance due to inadequate sample size. So, the efficacy analysis based on weekly average of SBM might be biased in favor of higher doses. It is more appropriate to asses the efficacy results based on the change from baseline.

This reviewer performed analysis of change of weekly average number of SBMs from baseline using Wilcoxon test. Results of this analysis showed that all doses were statistically significant from placebo at week 1 and week 2. But, they failed to achieve statistical significance at week 3. No differences between the low dose (24 μg) and middle dose (48 μg) were observed at week 2 and week 3. At week 1, middle dose (48 μg) was numerically slightly better than low dose (24 μg).

This reviewer performed post-hoc analyses for two stringent efficacy endpoints, the number of patients with increase of greater than or equal to one SBM/wk from baseline and number of patients with greater than or equal to 3 SBMs/wk. These efficacy endpoints were used for approval of Zelnorm. In the Zelnorm submission, complete spontaneous bowel movement (CSBM)/wk was assessed instead of SBM/wk.

The results of this reviewer's analyses for the number of patients with increase greater than or equal to one SBM/week and number of patients with greater than or equal to 3 SBMs/wk showed that all doses were numerically better than placebo for both two stringent endpoints and all timepoints (week 1, week 2, week 3, and over the period week 1 to week 3). But, they failed to achieve statistical significance due to insufficient sample sizes. Furthermore, the low dose (24 μg) was close to the middle dose (48 μg) at week 1, week 3, and week 1 to week 3. However, the low dose (24 μg) was slightly better than the middle dose (48 μg) at week 2. So, the minimum effective dose might be the low dose (24 μg). The low dose (24 μg) should be included in the Phase III studies.

2. INTRODUCTION

2.1 Overview

Ru-0211 is a unique prostaglandin metabolite analogue that is highly selective for ClC-2 chloride channels. Activation of these channels in the gastrointestinal tract increase Cl^- transport in the lumen, enhances fluid secretion into the bowel, and improves fecal transit.

The sponsor has submitted three adequate and well-controlled studies (SC9921, SC0131 and SC0232) to support the claim. Studies SC0131 and SC0232 were carried out using identical study design, and these trials are considered the pivotal studies for this submission. Study SC9921 was a Phase IIb dose-ranging study that utilized a similar study design.

2.2 Data Sources

The sponsor has submitted two pivotal Phase III studies and one Phase IIb dose ranging study for the claim. These studies include:

Study SC0232 – A double-blind, multi-center, randomized, placebo-controlled, phase III study of the efficacy and safety of oral RU-0211 for the treatment of occasional constipation.

Study SC0131 - A double-blind, multi-center, randomized, placebo-controlled, phase III study of the efficacy and safety of oral RU-0211 for the treatment of occasional constipation.

Study SC9921 - A double-blind, multi-center, randomized, placebo-controlled, phase IIb study of the efficacy and safety of oral RU-0211 for the treatment of chronic constipation.

All data were submitted in eCTD.

3. STATISTICAL EVALUATION

3.1 Evaluation of Efficacy

3.1.1 Study SC0232

3.1.1.1 Study Design

This study was a double-blind, randomized, multi-site (20 sites), placebo controlled study for the treatment of occasional constipation.

The objective of this study was to evaluate the efficacy and safety of 48 μg RU-0211 (24 μg RU-0211 b.i.d.) when compared with placebo for the treatment of occasional constipation.

Subjects with a documented history of constipation, defined as, on average, <3 SBMs per week as confirmed during the baseline/washout period and who were symptomatic for constipation were eligible for enrollment in the study.

This study consisted of a two week baseline/washout period, followed by a four week, randomized, double-blind, placebo-controlled treatment period and a follow-up visit 14 days after the end of treatment.

No rescue medications were allowed during Week 1 of treatment; however, the study design did allow for administration of rescue medication during both the baseline/washout period and during Treatment Weeks 2, 3, and 4 of treatment, but only as prescribed by the investigator. After 3 consecutive days of not having a spontaneous BM, the investigator might prescribe to the subject a 10 mg bisacodyl suppository. If this was not effective, a Fleet enema would be used. No rescue medication would be allowed in the 48 hours before randomization into the double-blind period.

Following initial assessment and after completing a 2-week baseline/washout, eligible subjects were randomly assigned to receive either RU-0211 24μg oral capsules twice daily (b.i.d.) or matching placebo b.i.d. for 4 weeks.

All subjects were to take 1 capsule (RU-0211·24 μg or placebo) 2 times each day (AM and PM doses, at breakfast and dinner with at least 8 ounces of water).

The study consisted of a screening visit (Visit 1, Days -15 to -2), an enrollment visit (Visit 2, Day -1), 2 interim visits (Visit 3, Day 8 and Visit 4, Day 15; these occurred after 1 and 2 weeks of treatment), an end of treatment visit (Visit 5, Day 29), and follow-up evaluation (Visit 6, Day 43) approximately 2 weeks after Visit 5.

Subjects were screened at Visit 1 to determine their eligibility to enroll in the trial. This visit was to take place approximately 14 days prior to the subject entering the treatment period and receiving study drug. The next visit (Visit 2) was scheduled for 2 weeks after the first day of the baseline/washout period. Subjects were considered eligible for randomization into treatment period if they had completed the 2-week baseline/washout period and they had demonstrated having constipation by recording, on average, less than 3 SBMs per week during the baseline/washout period. The next visit (Visit 3) was scheduled in approximately 8 days. The subject was instructed to complete the diary and bring diary to Visit 3.The next visit (Visit 4), a telephone interview, was scheduled in approximately 7 days. The subject was instructed to complete the diary and continue dosing study medication. Visit 5 was to take place approximately 14 days after Visit 4, approximately 4 weeks of treatment. Visit 6 was to take place approximately 14 days after Visit 5; a phone visit was conducted where the subject answered general questions.

Subjects were considered evaluable for efficacy if they were randomized and took at least 1 dose of double-blind study medication.

The "last observation carried forward" (LOCF) method would be used to impute missing values primary caused by early withdrawal from the study.

The primary efficacy variable was the frequency of SBMs at Week 1. An SBM was defined as any BM that did not occur within 24 hours after rescue medication use.

The secondary efficacy variables were as follows:

- Frequency of SBMs at Weeks 2, 3, and 4 and all bowel movements (BMs) at Weeks 1, 2, 3, and 4.
- Weekly responder rates
- Percentage of subjects with an SBM within 24 hours of first study drug administration
- Time to first SBM
- Weekly symptomatic assessment of average degree of stool consistency, average degree of straining, average degree of severity of constipation, global assessment of treatment effectiveness, and abdominal symptoms (bloating and discomfort).

At all visits except Visit 1, the severity of the subject's constipation over the past week was assessed using the following 5-point scale:

0=absent
1=mild
2=moderate
3=severe
4=very severe

At Visits 3, 4, 5, and 6, the treatment effectiveness over the past week was assessed using the following 5-point scale:

0=not at all effective
1=a little bit effective
2=moderate effective
3=quite a bit effective
4=extreme effective

At all visits, subjects were asked to evaluate abdominal symptoms (bloating and discomfort upon waking in the morning) using the following 5-point scale:

0=absent
1=mild
2=moderate
3=severe
4=very severe

The daily diary was completed during the 14 days immediately before Visit 2 and during the treatment period. Subjects who had a flexible sigmoidscopy with or without barium enema or a colonoscopy performed as part of the entrance criteria were instructed to start keeping their diary 1 week after the evaluative procedure or until their bowel habits had returned to "normal."

1. If a BM was produced, and if so
   a) date and time of BM
   b) consistency of BM
   c) degree of straining
2. Date, time, and type of any rescue medication used

Subjects evaluated BM consistency using the following 5-point scale:

0=very loose
1=loose
2=normal
3=hard
4=very hard (little balls)

Subject evaluated BM straining using the following 5-point scale:

0=no straining
1=mild straining
2=moderate straining
3=severe straining
4=very severe straining

Using a 2-sided Wilcoxon rank-sum test, and assuming a Week 1 difference between the treatment groups of 2 BMs and a standard deviation of 4.5, 116 subjects per treatment group were sufficient in order to reject the null hypothesis. Assuming a 3% dropout by Day 4 of the study, 120 subjects were proposed to be randomized into each treatment group.

3.1.1.2 Sponsor's Analysis

A total of 237 patients were randomized to treatment groups (119 in RU-0211 48 µg and 118 in placebo).

A total of 206 subjects (86.9%) completed the study. The most common reasons for discontinuation were AE (6.8%), lack of efficacy (3.0%), and lost to follow-up (2.12%).

Subjects in the RU-0211 48 µg discontinued more frequently (20, 16.8%) than placebo subjects (11, 9.3%). In general, subjects in the RU-0211 48 µg group appeared more likely to discontinue during Weeks 1 and 2.

Two hundred thirty-seven (237) subjects were randomized, received study drug, and made up the ITT data sets; and 206 subjects were randomized, received study drug, completed the entire study period, and made up the COM data set.

3.1.1.2.1 Planned Analysis

In order to adjust for early withdrawals, weekly SBM frequency rates were calculated as follows:

(Number of SBMs/Number of days) x 7

Where the number of days in the denominator is the number of days during the week that the subject was in the study. If the number of days was less than 4, then the data were considered insufficient and rate was missing.

Since far outliers are commonly observed in these data, parametric models may not be robust. Results from primary efficacy variable would therefore be analyzed by a van Elteren test stratified by center. Small centers would be pooled if necessary.

Frequency rate of SBMs at Weeks 2, 3, and 4 were analyzed as discussed above for Week 1. If the number of days for a given week was 0, then the last observation carried forward (LOCF) method was used to impute the frequency rate from the rate for the most recent week. However,

if the number of days in the week was less than 4, then the most recent data from days during the previous week were combined with data from the current week in order to bring the number of days up to 4.

Since previous results indicated very low correlations between baseline and post-baseline efficacy values, no adjustment such as change from baseline would be used in the analyses.

A longitudinal analysis of the frequency rates of SBMs and of all BMs was performed in order to assess the treatment effect over time. Missing values were not imputed for the analysis. The model included terms of treatment, time, pooled center, and baseline severity (weekly SBM rate during the baseline period). The time variable was defined by treatment Weeks, 1, 2, 3, and 4. Treatment-by-time and treatment-by-pooled center interactions were included in the model and tested one at a time at the=0.10 level.

This analysis was performed using either the SAS PROCedure MIXED or the generalized estimation equation approach (GEE). For either method, the most appropriate covariance structure would be applied and fitted to a model that would include factors for treatment, center, and week main effects. Two-way interactions with the treatment effect and a baseline score covariate would be included in the initial model and tested at the alpha=0.10 level.

In order to assess treatment response and to account for study dropout and rescue medication use, a trichtomous responder analysis was performed for each week.

A non-responder was defined as any subject with an SBM frequency rate of < 3 for a given week, any subject who dropped out during or prior to the given week due to lack of efficacy, or any subject who used rescue medication during or within 24 hours prior to the given week. If the subject dropped out for a reason other than lack of efficacy, did not use rescue medication, and had <4 days of diary data within the given week, then that subject's responder status would counted as missing for that week. Otherwise, the subject would be considered a responder. A responder with an SBM frequency rate ≥ 3 but < 4 was considered a moderate responder. Otherwise, the subject was a full responder (≥ 4).

All subjects who were randomized and took at least 1 dose of study medication made up the ITT population. Primary and secondary efficacy analyses were performed on this data set.

Subjects who completed the entire treatment period of the study made up Completers (COM) population. This data set was used for supporting analyses of efficacy.

Subjects who violated the inclusion/exclusion criteria or met any of the protocol deviation criteria were considered protocol violators for the applicable weeks and were removed from the PP subset at these weeks.

The frequency rate of SBMs during Week 1 was analyzed using a van Elteren test stratified by pooled center to test the null hypothesis of equal SBM rates between placebo and RU-0211 48 µg at the end of Week 1.

Frequency rate of SBMs and all BMs at Weeks 1, 2, 3 and 4 were analyzed using the same method as for the primary efficacy analysis. In addition, a longitudinal analysis of SBM and BM frequency rate was performed.

The number and percent of non-responders, moderate responders, and full responders were summarized by treatment group at each week using a van Elteren test stratified by pooled center.

The percentage of subjects with an SBM during the 24 hours since the first intake of study drug and by the time to the first SBM was analyzed using the Cochran-Mantel-Haenszel (CMH) test stratified by pooled center.

The comparison between treatment groups for the time to first SBM was displayed graphically with Kaplan-Meier curves, and a Cox proportional hazard model was used to analyze these data.

Average degree of stool consistency and average degree of straining were analyzed using van Elteren tests stratified by pooled center at Weeks 1, 2, 3, and 4.

Average degree of severity of constipation, global assessment of treatment effectiveness, and abdominal symptoms (bloating and discomfort) were analyzed using van Elteren tests stratified by pooled center at Weeks 1, 2, 3, and 4, and at follow-up.

Changes from baseline in efficacy variables were evaluated using the Wilcoxon signed-rank test.

3.1.1.2.2 Treatment Group Comparability

The summary of results of comparability of treatment groups at baseline for all randomized patients is given in Appendix Table 1.

As seen from Appendix Table 1, no statistically significant differences between the two treatment groups were observed for demographic and baseline characteristics with exception for baseline number of SBMs.

3.1.1.2.3 Sponsor's Analysis of Primary Efficacy Parameter

The primary efficacy parameter for this study was the SBM frequency rate at Week 1.

The summary of results of sponsor's analysis of primary efficacy variable is given below.

Summary of SBM Frequency Rates at Week 1
(Intent-to-Treat Population with LOCF)
Study SC0232

Week	Placebo n	Placebo Mean (St. Dev)	Placebo Median	RU-0211 48 µg n	RU-0211 48 µg Mean (St. Dev)	RU-0211 48 µg Median	P-value
Baseline	118	1.52 (0.801)	1.5	118	1.28 (0.881)	1.5	0.0126
Week 1	116	3.99 (2.706)	3.5	111	5.89 (4.022)	5.0	<0.0001

Copied from Table 11-3.
P-values are based on van Elteren tests adjusted for pooled center.

As seen from table above, the difference between the two treatment groups was statistically significant for SBM frequency rate at Week 1.

3.1.1.2.4 Sponsor's Analysis of Secondary Efficacy Parameter

The secondary efficacy parameters included frequency of SBMs at Weeks 2, 3, and 4 and all bowel movements (BMs) at Weeks 1, 2, 3, and 4, weekly responder rates, percentage of subjects with an SBM within 24 hours of first study drug administration, time to first SBM, weekly assessment of average degree of stool consistency, average degree of straining, average degree of severity of constipation, global assessment of treatment effectiveness, and abdominal symptoms (bloating and discomfort).

3.1.1.2.4.1 Frequency of SBMs at Weeks 2, 3, and 4

The summary of results of sponsor's analysis of frequency of SBMs at Weeks 2, 3, and 4 is given below.

Summary of SBM Frequency Rates at Weeks 2, 3, and 4
(Intent-to-Treat Population with LOCF)
Study SC0232

Week	Placebo n	Placebo Mean (St. Dev)	Placebo Median	RU-0211 48 µg n	RU-0211 48 µg Mean (St. Dev)	RU-0211 48 µg Median	P-value
Baseline	118	1.52 (0.801)	1.5	118	1.28 (0.881)	1.5	0.0126
Week 2	116	3.55 (2.670)	3.0	111	4.96 (4.208)	4.0	0.0487
Week 3	116	3.36 (2.755)	3.0	111	5.56 (4.560)	5.0	0.0004
Week 4	116	3.46 (2.861)	3.0	111	5.37 (4.804)	4.3	0.0068

Copied from Table 11-3.
P-values are based on van Elteren tests adjusted for pooled center.

As seen from table above, the differences were statistically significant at Weeks 2, 3, and 4.

Similar results were also observed for ITT subjects without LOCF, ITT subjects who completed the study, and PP subjects (see Appendix Table 2).

3.1.1.2.4.2 Frequency of BMs at Weeks 1, 2, 3, and 4

The summary of results of sponsor's analysis of frequency of BMs at Weeks 1, 2, 3, and 4 is given below.

Summary of BM Frequency Rates at Weeks 1, 2, 3, and 4
(Intent-to-Treat Population with LOCF)
Study SC0232

		Placebo			RU-0211 48 µg		
Week	n	Mean (St. Dev)	Median	n	Mean (St. Dev)	Median	P-value
Baseline	118	2.23 (1.135)	2.0	118	2.09 (1.095)	2.0	0.2397
Week 1	116	4.09 (2.669)	4.0	111	5.99 (3.956)	5.0	<0.0001
Week 2	116	4.00 (2.402)	4.0	111	5.32 (4.054)	4.0	0.0786
Week 3	116	3.99 (2.637)	3.1	111	5.92 (4.419)	5.0	0.0037
Week 4	116	3.92 (2.691)	3.2	111	5.65 (4.628)	5.0	0.0105

Copied from Table 11-5.
P-values are based on van Elteren tests adjusted for pooled center.

As seen from table above, at weeks 1, 2, 3, and 4, the mean and median BM frequency rates in the RU-0211 48 µg group were higher than the corresponding rates in the placebo group for ITT subjects with LOCF. The difference was statistically significant at Weeks 1, 3, and 4.

Similar results were also observed for ITT subjects without LOCF, ITT subjects who completed the study, and PP subjects (see Appendix Table 3).

3.1.1.2.4.3 Weekly Responder Rates

Weekly responder status for ITT subjects with LOCF is summarized below by treatment week.

Summary of Weekly Responder Status
(Intent-to-Treat Subjects with LOCF)
Study SC0232

Week [2]	Placebo (N = 118)	RU-0211 48 µg (N = 119)	P-value [3]
Week 1			
Full Responder	57/117 (48.7)	80/111 (72.1)	<0.0001
Moderate Responder	14/117 (12.0)	16/111 (14.4)	
Non-Responder	46/117 (39.3)	15/111 (13.5)	
Week 2			
Full Responder	50/117 (42.7)	64/111 (57.7)	0.0171
Moderate Responder	13/117 (11.1)	13/111 (11.7)	
Non-Responder	54/117 (46.2)	34/111 (30.6)	
Week 3			
Full Responder	42/117 (35.9)	68/111 (61.3)	0.0002
Moderate Responder	15/117 (12.8)	11/111 (9.9)	
Non-Responder	60/117 (51.3)	32/111 (28.8)	
Week 4			
Full Responder	45/117 (38.5)	66/111 (59.5)	0.0022
Moderate Responder	17/117 (14.5)	13/111 (11.7)	
Non-Responder	55/117 (47.0)	32/111 (28.8)	

LOCF: Missing values were imputed by the last observation carried forward.
[1] Full Responder was defined as responder with >= 4 SBMs per week;
Moderate Responder was defined as responder with >= 3 but < 4 SBMs per week;
Non-Responder was defined as subjects with < 3 SBMs for a given week, who dropped out during or prior to the given week due to lack of efficacy, or any subject who used rescue medication during or within 24 hours prior to the given week.
[2] The denominator represents the number of subjects with a non-missing responder status during the given week.
[3] P-values are based on van Elteren tests adjusted for pooled center.

As seen from table above, there was a significant difference between treatment groups in responder status at Weeks 1, 2, 3, and 4. In both treatment groups, the proportion of full responders was higher for Week 1 and gradually decreased over time. Generally, similar results were observed for ITT subjects without LOCF, ITT subjects who completed the study, and PP subjects (see Appendix Table 4). Of note, for both completers and PP subjects, the difference in the proportion of responders at Week 2 was not statistically significant (p=0.0588 for completers; p=0.0808 for PP). In both data sets, the proportion of responders at Week 2 in the RU-0211 48 µg was approximately 58% and the proportion in the placebo group was approximately 45-46%.

3.1.1.2.4.4 Percentage of Subjects with an SBM within 24 Hours of First Study Drug

Summaries of the number and percentage of subjects who had an SBM within 24 hours after the first administration of study drug are given in Appendix Table 5.

As seen from Appendix Table 5, the difference in SBM occurrence during the 24 hours after the first study drug administration between the treatment groups was statistically significant. Similar results were also observed for PP subjects.

3.1.1.2.4.5 Time to First SBM

Kaplan-Meier plot of the time to first SBM is given in Appendix Figure 6 for ITT subjects without LOCF.

As seen from Appendix Figure 6, onset of relief in the form of the first SBM was much faster in subjects treated with RU-0211 48 μg than among placebo subjects.

3.1.1.2.4.6 Average Degree of Stool Consistency

A summary of the average degree of stool consistency is given by treatment week for all subjects in Appendix Table 7.

As seen from Appendix Table 7, for ITT subjects with LOCF, the mean stool consistency reported in the RU-0211 48 μg group was lower than that in the placebo group, the difference was statistically significant at all time points. As shown in Appendix Table 7, similar results for the mean average degree of stool consistency were observed for ITT subjects without LOCF, ITT subjects who completed the study and PP subjects.

3.1.1.2.4.7 Average Degree of Straining

A summary of the average degree of straining is given by treatment week for all subjects in Appendix Table 8.

As seen from Appendix Table 8, for ITT subjects with LOCF, the mean average weekly degree of straining reported in the RU-0211 48 μg group was lower than that in the placebo group, the difference was statistically significant at all time points. As shown in Appendix Table 8, generally similar results for the mean average degree of straining consistency were observed for ITT subjects without LOCF, ITT subjects who completed the study and PP subjects. Notable difference was observed at Week 3: for ITT subjects without LOCF, the mean average degree of straining was 1.79 for placebo subjects and 1.43 for RU-0211 48 μg subjects (p=0.0529); and for ITT subjects who completed the study, the mean values were 1.76 and 1.43 (0.0673).

3.1.1.2.4.8 Average Severity of Constipation

A summary of the average constipation severity is given by treatment week for all subjects in Appendix Table 9.

As seen from Appendix Table 9, for ITT subjects with LOCF, the mean average weekly constipation severity reported in the RU-0211 48 μg group was lower than that in the placebo group, the difference was statistically significant at all time points. As shown in Appendix Table 9, generally similar results for the mean average constipation severity were observed for ITT subjects without LOCF, ITT subjects who completed the study and PP subjects. Notable difference was observed at Week 2: for ITT subjects who completed the study , the mean average severity of constipation was 1.95 for placebo subjects and 1.63 for RU-0211 48 μg subjects, and the result was not statistically significant (p=0.0544) .

3.1.1.2.4.9 Global Assessment of Treatment Effectiveness

A summary of treatment effectiveness is given by treatment week for all subjects in Appendix Table 10.

As shown in Appendix Table 10, for ITT subjects with LOCF, the mean treatment effectiveness reported in the RU-0211 48 μg group was higher than that in placebo group, the difference was statistically significant at all time points. As shown in Appendix Table 10, similar results for the mean treatment effectiveness were observed for ITT subjects without LOCF, ITT subjects who completed the study and PP subjects.

3.1.1.2.4.10 Abdominal Bloating

A summary of abdominal bloating is given by treatment week for all subjects in Appendix Table 11.

As seen from Appendix Table 11, for ITT subjects with LOCF, the mean level of abdominal bloating reported in the RU-0211 48 μg group was lower than that in the placebo group, but the difference was statistically significant only at Week 1 (p=0.0380). As shown in Appendix Table 11, similar results for the mean level of abdominal bloating were observed for ITT subjects without LOCF, ITT subjects who completed the study and PP subjects.

3.1.1.2.4.11 Abdominal Discomfort

A summary of abdominal discomfort is given by treatment week for all subjects in Appendix Table 12.

As seen from Appendix Table 12, for ITT subjects with LOCF, the mean level of abdominal discomfort reported in the RU-0211 48 μg group was lower than that in the placebo group, but the difference was not statistically significant at any time point. As shown in Appendix Table 12, similar results for the mean level of abdominal discomfort were observed for ITT subjects without LOCF, ITT subjects who completed the study and PP subjects.

3.1.1.3 Reviewer's Comments and Evaluation

3.1.1.3.1 Reviewer's Comments on Sponsor's ITT Population

The sponsor's ITT analysis was not true ITT analysis. It did not include all randomized patients. It excluded more patients in RU-0211 48 μg group than in placebo group (8 vs. 1, p=0.0180, chi-square test). So, sponsor's ITT analysis tends to be biased in favor of RU-0211 48 μg group.

3.1.1.3.2 Reviewer's Comments on Sponsor's Analysis of Primary Efficacy Variable

The pre-specified primary efficacy parameter was the SBM frequency rate at Week 1. The more clinically meaningful efficacy parameter should be the responder defined as a patient who had an

SBM frequency rate of ≥ 3 per week for all 4 weeks, and who did not use rescue medication during or within 24 hours prior to the given week, and who did not drop out during the study due to lack of efficacy. This endpoint is more stringent than the pre-specified primary endpoint. This reviewer performed the responder analysis. In this analysis, patients with the missing outcomes were set as no responders. The results from this analysis are given below.

Patients with ≥ 3 SBMs/wk for all 4 Weeks
Reviewer's ITT Population
Protocol SC0232

Analysis	RU-0211 48 μg	Placebo	Difference	P-value
With LOCF	61/119 (51.3%)	36/120 (30.0%)	21.3%	0.0010
Without LOCF	53/119 (44.5%)	34/120 (28.3%)	16.2%	0.0107

Compiled by this reviewer.
P-value was obtained by Fisher's Exact test.

As seen from table above, for more stringent efficacy endpoint (≥3 SBMs/wk for all 4 weeks), the RU-0211 48 μg was superior to the placebo for either analysis with LOCF or analysis without LOCF.

3.1.1.3.3 **Reviewer's Comment on Sponsor's Analysis on Weekly Responder Rates**

As stated in Section 3.1.1.3.1, the sponsor's analysis tends to be biased in favor of RU-0211 48 μg group. It was also found that more patients in RU-0211 48 μg group were imputed by LOCF than in placebo at Weeks 3 and 4 (8 in RU-0211 48 μg group and 2 in placebo group). This reviewer performed CMH (Cochran-Mantel-Haenszel) test using modified ridit scores for reviewer's ITT population without LOCF. In these analyses, patients with missing outcomes were set as no responders.

The summary of results of reviewer's analysis on weekly responder rates at Weeks 1, 2, 3, and 4 for reviewer's ITT population without LOCF is given below.

Summary of Weekly Responder Status
(Reviewer's ITT Population without LOCF)
Study SC0232

Week	Placebo (N=118)	RU-0211 48 µg (N=119)	p-value
Week 1			
Full Responder	57/118 (48.3%)	80/119 (67.2%)	0.0009
Moderate Responder	14/118 (11.9%)	16/119 (13.4%)	
Non-Responder	47/118 (39.8%)	23/119 (19.3%)	
Week 2			
Full Responder	50/118 (42.4%)	64/119 (53.8%)	0.0742
Moderate Responder	13/118 (11.0%)	12/119 (10.1%)	
Non-Responder	55/118 (46.6%)	43/119 (36.1%)	
Week 3			
Full Responder	42/118 (35.6%)	61/119 (51.3%)	0.0243
Moderate Responder	14/118 (11.9%)	10/119 (8.4%)	
Non-Responder	62/118 (52.5%)	48/119 (40.3%)	
Week 4			
Full Responder	45/118 (38.1%)	59/119 (49.6%)	0.0986
Moderate Responder	15/118 (12.7%)	12/119 (10.1%)	
Non-Responder	58/118 (49.2%)	48/119 (40.3%)	

Compiled by this reviewer.
P-values were obtained by Cochran-Mantel-Haenszel method using modified ridit scores.

As seen from table above, in this reviewer's analysis of weekly responder for reviewer's ITT population without LOCF, the proportion of full responders was higher for RU-0211 48 µg group than placebo group (about 19% at Week 1, 15% at Week 3 and 11% at Weeks 2 and 4). But, contrary to the sponsor's finding based on sponsor's ITT analysis with LOCF, treatment difference achieved statistical significance only at Week 1 and Week 3 at significance level of 0.05 without adjustment for multiplicity.

3.1.1.3.4 **Subgroup Analysis**

Subgroup analyses were performed on the number of patients who were full responders or moderate responders for all 4 weeks by age, gender, flexible sigmoidoscopy, barium enema, colonoscopy, irritable bowel syndrome, and GERD

In these analyses, patients with missing outcomes were set treatment failures. The results of subgroup analyses of the number of patients with treatment success are given below.

Number of Patients who were Full Responders or Moderate Responders

For All 4 Weeks by Subgroup

Reviewer's ITT Population with LOCF
Protocol SC0232

Subgroup	RU-0211 48µg	Placebo	Difference	95% C. I.
Gender				
Male	10/15 (67%)	3/13 (23%)	44%	(10.5%, 76.7%)
Female	51/104 (49%)	33/105 (31%)	18%	(4.5%, 307%)
Age				
18 to 64	59/109 (54%)	33/108 (31%)	23%	(10.8%, 36.3%)
≥ 65	2/10 (20%)	3/10 (30%)	-10%	(-47.7%, 27.7%)
Flexible Sigmoidoscopy				
No	48/99 (49%)	30/91 (33%)	16%	(1.7%, 29.3%)
Yes	13/20 (65%)	6/27 (22%)	43%	(16.6%, 68.9%)
Barium Enema				
No	60/116 (52%)	36/115 (31%)	21%	(8.0%, 32.9%)
Yes	1/3 (33%)	0/3 (0.0%)	33%	(-20.0%, 86.7%)
Colonoscopy				
No	13/20 (65%)	6/25 (24%)	41%	(14.2%, 67.8%)
Yes	48/99 (49%)	30/93 (32%)	17%	(2.5%, 29.9%)
Irritable Bowel Syndrome				
No	58/106 (55%)	29/98 (30%)	25%	(12.0%, 38.2%)
Yes	3/13 (23%)	7/20 (35%)	-12%	(-42.9%, 19.1%)
Gastroesophogeal Reflux Disease				
No	40/85 (47%)	23/84 (27%)	20%	(5.4%, 33.9%)
Yes	21/34 (62%)	13/34 (38%)	24%	(0.4%, 46.6%)

Compiled by this reviewer.

As seen from table above, treatment difference was consistent among all subgroups with exception for irritable bowel syndrome. Interaction between treatment and subgroup was found to be statistically significant at significant level of 0.10 for subgroup of irritable bowel syndrome with p-value 0.0496 (Breslow-Day method).

3.1.2 Study SC0131

3.1.2.1 Study Design

The study design for this study was similar to those for Study SC0132 with few exceptions listed below.

The secondary efficacy variables did not include all bowel movements (BMs) at Weeks 1, 2, 3, and 4.

3.1.2.2 Sponsor's Analysis

A total of 244 patients were randomized to treatment groups (120 in RU-0211 48 µg and 124 in placebo). Two subjects (0512 and 0609) in the placebo group were randomized but not treated.

A total of 224 subjects (92.6%) completed the study. The most common reasons for discontinuation were AE (4.1%), voluntary withdrawal (1.6%), lack of efficacy (1.2%), and lost to follow-up (1.2%).

More subjects in the RU-0211 48 µg discontinued (14, 11.7%) than placebo subjects (4, 3.3%) (p=0.0129). In general, subjects in the RU-0211 48 µg group appeared more likely to discontinue during Weeks 1 and 2.

Two hundred forty-two (242) subjects were randomized, received study drug, and made up the ITT data sets; and 222 subjects were randomized, received study drug, completed the entire study period, and made up the COM data set.

3.1.2.2.1 Planned Analysis

It is the same as in Section 3.1.1.2.1.

3.1.2.2.2 Treatment Group Comparability

The summary of results of comparability of treatment groups at baseline for all randomized patients is given in Appendix Table 13.

As seen from Appendix Table 13, no statistically significant differences between the two treatment groups were observed for demographic and baseline characteristics.

3.1.2.2.3 Sponsor's Analysis of Primary Efficacy Parameter

The primary efficacy parameter for this study was the SBM frequency rate at Week 1. An SMB was defined as any bowel movement that did not occur within 24 hours after rescue medication use.

The summary of results of sponsor's analysis of primary efficacy variable is given below.

Summary of SBM Frequency Rates at Week 1
(Intent-to-Treat Population with LOCF)
Study SC0131

Week	Placebo n	Placebo Mean (St. Dev)	Placebo Median	RU-0211 48 µg n	RU-0211 48 µg Mean (St. Dev)	RU-0211 48 µg Median	P-value
Baseline	119	1.47 (1.325)	1.5	120	1.37 (0.873)	1.5	0.6120
Week 1	122	3.46 (2.285)	3.0	116	5.69 (4.417)	5.0	0.0001

Copied from Table 14.2.1.1
P-values are based on van Elteren tests adjusted for pooled center.

As seen from table above, the difference between the two treatment groups was statistically significant for SBM frequency rate at Week 1.

Similar results were also observed for ITT subjects without LOCF, ITT subjects who completed the study, and PP subjects (see Appendix Table 14).

3.1.2.2.4 Sponsor's Analysis of Secondary Efficacy Parameter

The secondary efficacy parameters included frequency of SBMs at Weeks 2, 3, and 4 and all bowel movements (BMs) at Weeks 1, 2, 3, and 4, weekly responder rates, percentage of subjects with an SBM within 24 hours of first study drug administration, time to first SBM, weekly assessment of average degree of stool consistency, average degree of straining, average degree of severity of constipation, global assessment of treatment effectiveness, and abdominal symptoms (bloating and discomfort).

3.1.2.2.4.1 Frequency of SBMs at Weeks 2, 3, and 4

The summary of results of sponsor's analysis of frequency of SBMs at Weeks 2, 3, and 4 is given below.

Summary of SBM Frequency Rates at Weeks 2, 3, and 4
(Intent-to-Treat Population with LOCF)
Study SC0131

Week	Placebo n	Placebo Mean (St. Dev)	Placebo Median	RU-0211 48 µg n	RU-0211 48 µg Mean (St. Dev)	RU-0211 48 µg Median	P-value
Baseline	119	1.47 (1.325)	1.5	120	1.37 (0.873)	1.5	0.6120
Week 2	122	3.18 (2.530)	3.0	116	5.06 (4.076)	4.0	0.0017
Week 3	122	2.84 (2.231)	2.0	116	5.25 (4.875)	5.0	0.0002
Week 4	122	2.91 (2.357)	2.3	116	5.30 (4.735)	4.0	0.0002

Copied from Table 14.2.1.1
P-values are based on van Elteren tests adjusted for pooled center.

As seen from table above, the differences were statistically significant at Weeks 2, 3, and 4.

Similar results were also observed for ITT subjects without LOCF, ITT subjects who completed the study, and PP subjects (see Appendix Table 14).

3.1.2.2.4.2 Frequency of BMs at Weeks 1, 2, 3, and 4

The summary of results of sponsor's analysis of frequency of BMs at Weeks 1, 2, 3, and 4 is given below.

Summary of BM Frequency Rates at Weeks 1, 2, 3, and 4
(Intent-to-Treat Population with LOCF)
Study SC0131

	Placebo			RU-0211 48 µg			
Week	n	Mean (St. Dev)	Median	n	Mean (St. Dev)	Median	P-value
Baseline	119	2.50 (1.703)	2.0	120	2.28 (1.131)	2.0	0.5980
Week 1	122	3.71 (2.291)	3.0	116	5.80 (4.326)	5.0	0.0002
Week 2	122	3.71 (2.452)	3.0	116	5.59 (3.745)	5.0	<0.0001
Week 3	122	3.50 (2.254)	3.0	116	5.76 (4.624)	5.0	0.0037
Week 4	122	3.58 (2.260)	3.0	116	6.02 (4.548)	5.4	<0.0001

Copied from Table 14-2.2.1
P-values are based on van Elteren tests adjusted for pooled center.

As seen from table above, at Weeks 1, 2, 3, and 4, the mean and median BM frequency rates in the RU-0211 48 µg were higher than the corresponding rates in the placebo group for ITT subjects with LOCF. The difference was statistically significant at Weeks 1, 2, 3, and 4.

Similar results were also observed for ITT subjects without LOCF, ITT subjects who completed the study, and PP subjects (see Appendix Table 15).

3.1.2.2.4.3 Weekly Responder Rates

Weekly responder status for ITT subjects with LOCF is summarized below by treatment week.

Summary of Weekly Responder Status
(Intent-to-Treat Subjects with LOCF)
Study SC0131

Week	Placebo (N=122)	RU-0211 48 µg (N=116)	p-value
Week 1			
Full Responder	53/122 (43.4%)	75/116 (64.7%)	0.0023
Moderate Responder	19/122 (15.6%)	14/116 (12.1%)	
Non-Responder	50/122 (41.0%)	27/116 (23.3%)	
Week 2			
Full Responder	44/122 (36.1%)	67/116 (57.8%)	0.0037
Moderate Responder	17/122 (13.9%)	10/116 (8.6%)	
Non-Responder	61/122 (50.0%)	39/116 (33.6%)	
Week 3			
Full Responder	35/122 (28.7%)	65/116 (56.0%)	0.0003
Moderate Responder	16/122 (13.1%)	8/116 (6.9%)	
Non-Responder	71/122 (58.2%)	43/116 (37.1%)	
Week 4			
Full Responder	34/122 (27.9%)	67/116 (57.8%)	<0.0001
Moderate Responder	20/122 (16.4%)	10/116 (8.6%)	
Non-Responder	68/122 (55.7%)	39/116 (33.6%)	

Copied from Table 14.2.4.1

As seen from table above, there was a significant difference between treatment groups in responder status at Weeks 1, 2, 3, and 4. In both treatment groups, the proportion of full responders was higher for Week 1.

Similar results were observed for ITT subjects without LOCF, ITT subjects who completed the study, and PP subjects (see Appendix Table 16).

3.1.2.2.4.4 Percentage of Subjects with an SBM within 24 Hours of First Study Drug

Summaries of the number and percentage of subjects who had an SBM within 24 hours after the first administration of study drug are given in Appendix Table 17.

As seen from Appendix Table 17, the difference in SBM occurrence during the 24 hours after the first study drug administration between the treatment groups was statistically significant. Similar results were also observed for PP subjects.

3.1.2.2.4.5 Time to First SBM

Kaplan-Meier plot of the time to first SBM is given in Appendix Figure 18 for ITT subjects without LOCF.

As seen from Appendix Figure 18, onset of relief in the form of the first SBM was much faster in subjects treated with RU-0211 48 μg than among placebo subjects.

3.1.2.2.4.6 Average Degree of Stool Consistency

A summary of the average degree of stool consistency is given by treatment week for all subjects in Appendix Table 19.

As seen from Appendix Table 19, for ITT subjects with LOCF, the mean stool consistency reported in the RU-0211 48 μg group was lower than that in the placebo group, the difference was statistically significant at all time points. As shown in Appendix Table 19, similar results for the mean average degree of stool consistency were observed for ITT subjects without LOCF, ITT subjects who completed the study and PP subjects.

3.1.2.2.4.7Average Degree of Straining

A summary of the average degree of straining is given by treatment week for all subjects in Appendix Table 20.

As seen from Appendix Table 20, for ITT subjects with LOCF, the mean average weekly degree of straining reported in the RU-0211 48 μg group was lower than that in the placebo group, the difference was statistically significant at all time points. As shown in Appendix Table 20, generally similar results for the mean average degree of straining consistency were observed for ITT subjects without LOCF, ITT subjects who completed the study and PP subjects. Notable difference was observed at Week 3: for ITT subjects without LOCF, the mean average degree of straining was 1.79 for placebo subjects and 1.43 for RU-0211 48 μg subjects (p=0.0529); and for ITT subjects who completed the study, the mean values were 1.76 and 1.43 (0.0673).

3.1.2.2.4.8 Average Severity of Constipation

A summary of the average constipation severity is given by treatment week for all subjects in Appendix Table 21.

As seen from Appendix Table 21, for ITT subjects with LOCF, the mean average weekly constipation severity reported in the RU-0211 48 μg group was lower than that in the placebo group, the difference was statistically significant at all time points. As shown in Appendix Table 21, generally similar results for the mean average constipation severity were observed for ITT subjects without LOCF, ITT subjects who completed the study and PP subjects. Notable difference was observed at Week 2: for ITT subjects who completed the study , the mean average severity of constipation was 1.95 for placebo subjects and 1.63 for RU-0211 48 μg subjects, and the result was not statistically significant (p=0.0544) .

3.1.2.2.4.9 Global Assessment of Treatment Effectiveness

A summary of treatment effectiveness is given by treatment week for all subjects in Appendix Table 22.

As seen from Appendix Table 22, for ITT subjects with LOCF, the mean treatment effectiveness reported in the RU-0211 48 μg group was higher than that in the placebo group, the difference was statistically significant at all time points. As shown in Appendix Table 22, similar results for the mean treatment effectiveness were observed for ITT subjects without LOCF, ITT subjects who completed the study and PP subjects.

3.1.2.2.4.10 Abdominal Bloating

A summary of abdominal bloating is given by treatment week for all subjects in Appendix Table 23.

As seen from Appendix Table 23, for ITT subjects with LOCF, the mean level of abdominal bloating reported in the RU-0211 48 μg group was lower than that in the placebo group, but the difference was statistically significant only at Week 1 (p=0.0380). As shown in Appendix Table 23, similar results for the mean level of abdominal bloating were observed for ITT subjects without LOCF, ITT subjects who completed the study and PP subjects.

3.1.2.2.4.11 Abdominal Discomfort

A summary of abdominal discomfort is given by treatment week for all subjects in Appendix Table 24.

As seen from Appendix Table 24, for ITT subjects with LOCF, the mean level of abdominal discomfort reported in the RU-0211 48 μg group was lower than that in the placebo group, but the difference was not statistically significant at any time point. As shown in Appendix Table 24, similar results for the mean level of abdominal discomfort were observed for ITT subjects without LOCF, ITT subjects who completed the study and PP subjects.

3.1.2.3 Reviewer's Comments and Evaluation

3.1.2.3.1 Reviewer's Comments on Sponsor's ITT Population

The sponsor's ITT analysis was not true ITT analysis. It did not include all randomized patients. It excluded more patients in RU-0211 48 μg group than in placebo group (4 vs. 0). So, sponsor's ITT analysis might tend to be biased in favor of RU-0211 48 μg group.

3.1.2.3.2 Reviewer's Comments on Sponsor's Analysis of Primary Efficacy Variable

The pre-specified primary efficacy parameter was the SBM frequency rate at Week 1. The more clinically meaningful efficacy parameter should be the responder defined as a patient who had an SBM frequency rate of ≥ 3 per week for all 4 weeks, and who did not use rescue medication during or within 24 hours prior to the given week, and who did not drop out during the study due to lack of efficacy. This endpoint is more stringent than the pre-specified primary endpoint. This reviewer performed the responder analysis. In this analysis, the missing outcomes were set to as no responder. The results from this analysis are given below.

Patients with ≥ 3 SBMs/wk for all 4 Weeks
Reviewer's ITT Population
Protocol SC0131

Analysis	RU-0211 48 µg	Placebo	Difference	P-value
With LOCF	54/120 (45.0%)	26/122 (21.3%)	23.7%	0.0001
Without LOCF	49/120 (40.8%)	26/122 (21.3%)	19.5%	0.0013

Compiled by this reviewer.
P-value was obtained by Fisher's Exact test.

As seen from table above, for more stringent efficacy endpoint (≥ 3 SBMs/wk for all 4 weeks), the RU-0211 48 µg was superior to the placebo for either analysis with LOCF or analysis without LOCF.

3.1.2.3.3 **Reviewer's Comment on Sponsor's Analysis on Weekly Responder Rates**

As stated in Section 3.1.2.3.1, the sponsor's analysis tends to be biased in favor of RU-0211 48 µg group. It was also found that more patients in RU-0211 48 µg group were imputed by LOCF than in placebo at Weeks 3 and 4 (8 in RU-0211 48 µg group and 2 in placebo group). This reviewer performed CMH (Cochran-Mantel-Haenszel) test using modified ridit scores for reviewer's ITT population without LOCF. In these analyses, patients with missing outcomes were set as no responders.

The summary of results of reviewer's analysis on weekly responder rates at Weeks 1, 2, 3, and 4 for reviewer's ITT population without LOCF is given below.

Summary of Weekly Responder Status
(Reviewer's ITT Population without LOCF)
Study SC0131

Week	Placebo (N=122)	RU-0211 48 µg (N=120)	p-value
Week 1			
Full Responder	53/122 (43.4%)	75/120 (62.5%)	0.0031
Moderate Responder	19/122 (15.6%)	14/120 (11.7%)	
Non-Responder	50/122 (41.0%)	31/120 (25.8%)	
Week 2			
Full Responder	44/122 (36.1%)	66/120 (55.0%)	0.0077
Moderate Responder	17/122 (13.9%)	10/120 (8.3%)	
Non-Responder	61/122 (50.0%)	44/120 (36.7%)	
Week 3			
Full Responder	35/122 (28.7%)	62/120 (51.7%)	0.0014
Moderate Responder	16/122 (13.1%)	8/120 (6.7%)	
Non-Responder	71/122 (58.2%)	50/120 (41.7%)	
Week 4			
Full Responder	34/122 (27.9%)	62/120 (51.7%)	0.0012
Moderate Responder	20/122 (16.4%)	10/120 (8.3%)	
Non-Responder	68/122 (55.7%)	48/120 (40.0%)	

Compiled by this reviewer.
P-values were obtained by Cochran-Mantel-Haenszel method using modified ridit scores.

As seen from table above, in this reviewer's analysis of weekly responder for reviewer's ITT population without LOCF, the proportion of full responders was higher for RU-0211 48 µg group than placebo group (about 19% at Weeks 1 and 2, 23% at Weeks 3 and 4). Treatment difference was statistically significant fat Weeks 1 thro 4 at significance level of 0.05 without adjustment for multiplicity.

3.1.2.3.4 Subgroup Analysis

Subgroup analyses were performed on the number of patients who were full responders or moderate responders for all 4 weeks by age, gender, flexible sigmoidoscopy, barium enema, colonoscopy, irritable bowel syndrome, and GERD

In these analyses, patients with missing outcomes were set treatment failures. The results of subgroup analyses of the number of patients with treatment success are given below.

Number of Patients who were Full Responders or Moderate Responders

For All 4 Weeks by Subgroup

Reviewer's ITT Population with LOCF
Protocol SC0131

Subgroup	RU-0211 48μg	Placebo	Difference	95% C. I.
Gender				
Male	6/13 (46%)	3/12 (25%)	21%	(-15.4%, 57.7%)
Female	48/107 (45%)	23/110 (21%)	24%	(11.8%, 36.1%)
Age				
18 to 64	45/107 (42%)	22/103 (21%)	21%	(8.4%, 33.0%)
≥ 65	9/13 (69%)	4/19 (21%)	48%	(17.1%, 79.3%)
Flexible Sigmoidoscopy				
No	38/82 (46%)	20/85 (24%)	22%	(8.7%, 36.9%)
Yes	16/38 (42%)	6/37 (16%)	26%	(6.2%, 45.6%)
Barium Enema				
No	50/111 (45%)	22/111 (20%)	25%	(13.4%, 37.1%)
Yes	4/9 (44%)	4/11 (36%)	8%	(-35.1%, 51.2%)
Colonoscopy				
No	16/38 (42%)	6/35 (17%)	25%	(4.9%, 45.0%)
Yes	38/82 (46%)	20/87 (23%)	23%	(9.4%, 37.3%)
Irritable Bowel Syndrome				
No	40/88 (46%)	24/96 (25%)	21%	(6.9%, 34.0%)
Yes	14/32 (44%)	2/26 (8%)	36%	(16.0%, 56.1%)
Gastroesophogeal Reflux Disease				
No	37/84 (44%)	19/88 (22%)	22%	(8.8%, 36.1%)
Yes	17/36 (47%)	7/34 (21%)	26%	(5.4%, 47.9%)

Compiled by this reviewer.

As seen from table above, treatment difference was consistent among all subgroups.

3.1.3 Study SC9921

3.1.3.1 Study Design

This study was a double-blind, randomized, multi-center (8 centers), placebo controlled study for the treatment of occasional constipation.

The objective of this study was to assess the efficacy and safety of different dose regimens of oral RU-0211 (24 μg, 48 μg, and 74 μg) compared to placebo on relief of chronic constipation as assessed by the number of spontaneous bowel movements and abdominal symptoms.

Chronic constipation was identified as < 3 SBMs per week, on average, accompanied by at least 1 symptom of constipation for at least 6 months.

The duration of treatment was 21 days. The study consisted of a screen visit (Visit 1, Day -14), a randomization visit (Visit 2, Day -1), 2 interim visits (Visit 3, Day 8 and Visit 4, Day 15), and an end of treatment visit (Visit 5, Day 22).

The primary efficacy endpoint was daily average number of SBMs.

The secondary efficacy endpoints were:

1). Percentage of patients with a SBMs on Day1
2). Average degree of evacuation
3). Average degree of straining
4). Average stool consistency
5). Assessments of abdominal bloating and discomfort
6). Global assessment of constipation
7). Global assessment of treatment effectiveness
8). Usage of rescue medication
9). Percentage of patients using the rescue medication
10). Percentage of treatment failure

All randomized subjects who tool at least one dose of study drug constituted the population of the "intent-to-treat" population.

For all inferential analyses of efficacy, the between-group comparisons were performed between the placebo group and each of the RU-0211 groups.

Multiple treatment comparisons and repeated measurements analyses would be adjusted by Shaffer's modified sequentially rejective procedure in the following way:

The global alpha level will be protected by Shaffer's modified sequentially rejective testing procedure: First, the appropriate overall test with three degree of freedom. for the three contrasts of interest will be used to reject the hypothesis that none of them is significant. If this test is significant at alpha=5%, the three comparisons to placebo will conducted based adjusted alpha of 0.025. If any of the three p-values is lower than 0.025, the pertinent null hypothesis will be rejected at an experiment-wise alpha of 0.05, further testing of the other two hypothesis can be undertaken at a nominal alpha of 0.05 (Shaffer 1986).

The study was designed to detect an average difference of 2.5 bowel movements per week between RU-0211 and placebo. The standard deviation of this difference was estimated to be 2.6. In order to detect this difference with 86% power, approximately 25 patients per treatment group were needed. The power would be 0.86 with 25 patients per arm at alpha=0.025. These calculations were based on a noncentral t-distribution with α=0.025 in order to obtain an overall significance level of ≤ 0.05 in the multiple comparison. Assuming of 20% of subjects would not qualify based on the washout results, at least 30 subjects should be randomized into each treatment group.

3.1.3.2 **Sponsor's Analysis**

A total of 129 patients were randomized into the study: 33 patients in the placebo, 30 patients in the RU-0211 24 μg group, 32 patients in the RU-0211 48 μg group, and 34 patients in the RU-0211 72 μg group.

One patient each in the RU-0211 24 μg and 72 μg groups was randomized but not treated, making a total of 127 patients who were treated.

A total of 109 patients completed the study (26 for 24 μg, 27 for 48 μg, 28 for 72 μg and 28 for placebo). Withdrawal because of AEs was most common in RU-0211 48 μg group (5/32, 16%)

3.1.3.2.1 **Planned Analysis**

Because the assumption of constant variance was violated, the parametric methods planned for the analysis of bowel movement frequency data were replaced with nonparametric methods. To test for overall treatment effects, ANOVA and ANCOVA models were replaced with CMH tests stratified by investigator using modified ridit scores. To perform pairwise comparisons while adjusting for multiple comparisons, Dunnet's test was replaced with van Elteren's test, using Shaffer's modified sequentially rejective procedure to determine statistical significance.

The sponsor performed the following supplemental analyses after the clinical database was locked, and they were not included the original protocol or the statistical analysis plan.

These supplemental analyses were:

1). Weekly average number of bowel movement and spontaneous bowel movements
2). Responders and spontaneous responders
3). Treatment failures by week
4). Global and abdominal assessment over the double-blind period
5). Number of days with bowel movements
6). Change from baseline analyses

3.1.3.2.2 **Treatment Group Comparability**

The summary of results of comparability of treatment groups at baseline for all randomized patients is given in Appendix Table 25.

As seen from Appendix Table 25, no statistically significant differences among treatment groups were observed for demographic and baseline characteristics.

3.1.3.2.3 **Sponsor's Analysis of Primary Efficacy Parameter**

The primary efficacy variable was defined as the daily average number of spontaneous bowel movements per week. A summary of the daily average number of spontaneous bowel movements in the ITT population is given below.

Summary of Daily Average Number of SBMs at Baseline and Changes from Baseline Intent-to-Treat Subjects Study SC9921

		Placebo N=33	RU-0211 24 µg N=29	RU-0211 48 µg N=32	RU-0211 72 µg N=33	Among group p-value*
Baseline	n	31	29	32	32	0.241
	Mean (Std)	0.20 (0.133)	0.16 (0.130)	0.20 (0.117)	0.19 (0.127)	
Week 1	n	32	28	27	30	0.006
	Mean (Std)	0.56 (0.616)	0.76 (0.556)	0.97 (0.603)	0.87 (0.501)	
	p-value		0.253	0.002	0.003	
Week 2	n	28	27	26	30	0.014
	Mean (Std)	0.51 (0.518)	0.77 (0.671)	0.82 (0.643)	0.83 (0.555)	
	P-value		0.013	0.020	0.007	
Week 3	n	26	25	25	27	0.298
	Mean (Std)	0.58 (0.604)	0.72 (0.744)	0.74 (0.487)	0.81 (0.567)	
	p-value		0.234	0.099	0.197	

*P-value is based on CMH test, stratified by investigator using modified ridit scores testing the hypothesis of no differences among treatment group.
Copied from Table 11.4.1.1.1.

As seen from table above, at Week 1, the overall difference was statistically significant. Pairwise comparisons revealed statistically significant differences in favor of RU-0211 48 µg over placebo and in favor of RU-0211 72 µg over placebo. At Week 2, there was a clear dose-dependent increase in the mean daily average number of SBMs. The overall difference was statistically significant. Pairwise comparisons revealed statistically significant differences in favor of RU-0211 24 µg over placebo, in favor of RU-0211 48 µg over placebo and in favor of RU-0211 72 µg over placebo. At Week 3, the overall difference was not statistically significant.

3.1.3.3 Reviewer's Comments and Evaluation

3.1.3.3.1 Reviewer's Re-analysis of Weekly Average Number of SBMs

In the Study SC9921, the primary efficacy endpoint was pre-specified as number of (SBMs. The primary time point was pre-specified as Week 3 in the protocol. There was slight imbalance in weekly average number of SBMs at baseline (1.1 for 24 µg, 1.3 to 1.4 for other groups). It failed to achieve statistical significance due to inadequate sample size. So, the efficacy analysis based on weekly average of SBM might be biased in favor higher doses. It is more appropriate to asses the efficacy results based on the change from baseline. Summary of change of weekly average number of SBMs from baseline is given below.

Summary of Weekly Average Number of SBMs at Baseline and Changes from Baseline Intent-to-Treat Subjects Study SC9921

		Placebo N=33	RU-0211 24 µg N=29	RU-0211 48 µg N=32	RU-0211 72 µg N=33	Among group p-value
Baseline	n	31	29	32	32	
	Mean (Std)	1.41 (0.93)	1.11 (0.91)	1.39 (0.82)	1.30 (0.89)	
Week 1	n	30	28	27	29	0.0052
	Mean (Std)	2.64 (4.26)	4.31 (3.98)	5.39 (4.20)	4.68 (3.35)	
	p-value†		0.0263	0.0043	0.0044	
Week 2	n	26	27	26	29	0.0480
	Mean (Std)	2.10 (3.78)	4.36 (4.72)	4.34 (4.74)	4.39 (3.77)	
	p-value†		0.0190	0.0514	0.0197	
Week 3	n	25	25	25	26	0.1753
	Mean (Std)	2.55 (4.11)	4.05 (5.40)	3.90 (3.65)	4.28 (3.75)	
	p-value†		0.1075	0.1095	0.0523	

†Pairwise p-value with placebo was obtained by Wilcoxon test.
Among group p-value was obtained by Kruskal-Wallis test.
Prepared by this reviewer.

As seen from table above, all doses were statistically significant from placebo at Week 1 and Week 2. But, they failed to achieve statistical significance at Week 3, the primary time point pre-specified in the protocol. No differences between the low dose (24 µg) and middle dose (48 µg) were observed at Week 2 and Week 3. At Week 1, middle dose (48 µg) was numerically slightly better than low dose (24 µg).

3.1.3.3.2 **Reviewer's Post-hoc Analyses**

This reviewer performed post-hoc analyses for two stringent efficacy endpoints: the number of patients with increase of greater than or equal to one SBM/wk from baseline and number of patients with greater than or equal 3 to SBMs/wk. These efficacy endpoints were used for approval of Zelnorm. In the Zelnorm submission, CSBM/wk was assessed instead of SBM/wk.

The results of this reviewer's analyses for the number of patients with increase greater than or equal to one SBM/week and number of patients with greater than or equal to 3 SBMs/wk are given below.

Patients with Inc ≥ 1 SBM/wk
Intent-to-Treat Subjects
Study SC9921

		Placebo N=33	RU-0211 24 µg N=29	RU-0211 48 µg N=32	RU-0211 72 µg N=33	Among group p-value
Week 1	n	22 (66.7%)	23 (79.3%)	24 (75.0%)	24 (72.7%)	0.6729
	p-value†		0.3929	0.5874	0.7893	
Week 2	n	17 (51.5%)	23 (79.3%)	20 (62.5%)	24 (72.7%)	0.1878
	p-value†		0.0236	0.4553	0.1271	
Week 3	n	17 (51.5%)	18 (62.1%)	19 (59.4%)	21 (63.6%)	0.3741
	p-value†		0.4499	0.6203	0.4553	
Week 1 to Week 3	n	10 (30.3%)	16 (55.2%)	16 (50.0%)	18 (54.5%)	0.0783
	p-value†		0.0711	0.1324	0.0804	

†Pairwise p-value with placebo was obtained by Fisher's Exact test.
Among group p-value was obtained by Mantel-Haenszel test.
Prepared by this reviewer.

Patients with ≥ 3 SBMs/wk
Intent-to-Treat Subjects
Study SC9921

		Placebo N=33	RU-0211 24 µg N=29	RU-0211 48 µg N=32	RU-0211 72 µg N=33	Among group p-value
Week 1	n	20 (60.6%)	22 (75.9%)	22 (68.8%)	24 (72.7%)	0.4025
	p-value†		0.2777	0.6059	0.4339	
Week 2	n	16 (48.5%)	21 (72.4%)	20 (62.5%)	22 (66.7%)	0.2244
	p-value†		0.0719	0.3213	0.2127	
Week 3	n	15 (45.5%)	18 (62.1%)	18 (56.3%)	20 (60.6%)	0.2963
	p-value†		0.2130	0.4603	0.3240	
Week 1 to Week 3	n	9 (27.3%)	15 (51.7%)	15 (46.9%)	16 (48.5%)	0.1217
	p-value†		0.0683	0.1271	0.1271	

†Pairwise p-value with placebo was obtained by Fisher's Exact test.
Among group p-value was obtained by Mantel-Haenszel test.
Prepared by this reviewer.

As seen from tables above, all doses were numerically better than placebo for either of two stringent endpoints and each of timepoints (Week 1, Week 2, Week 3, and Week 1 to Week 3). But, they failed to achieve statistical significance due to insufficient sample sizes. Furthermore, the low dose (24 µg) was close to the middle dose (48 µg) at Week 1, Week 3, and Week 1 to Week 3. However, the low dose ((24 µg) was slightly better the middle dose (48 µg) at Week 2.

So, the minimum effective might be the low dose (24 μg). The low dose (24 μg) should be included in the Phase III studies.

3.2 Evaluation of Safety

This reviewer pooled two phase III studies (SC0232 and SC0131) for safety. Summary of results is given below.

Pooled Studies SC0232 and SC0131

Variable	RU-0211 48 μg (n=239)	Placebo (n=240)	Diff	Relative Risk	P-value
At least 1 AE	149 (62.3%)	103 (42.9%)	19.4%	1.5	<0.0001
At least 1 Treatment-related AE	112 (46.9%)	45 (18.9%)	28.0%	2.5	<0.0001
Withdrew Due to AE	24 (10.0%)	2 (0.8%)	9.2%	12.5	<0.0001

Complied by this reviewer.
P-value was obtained by CMH adjusted for study.

As seen from table above, the relative risk for RU-0211 48 μg compared to placebo was about 2.5 for patients with at least 1 treatment related AE. The relative risk for patients who withdrew due to AE was about 12.5.

For Phase IIb, the sponsor performed only one dose finding study SC9921 with doses of 24 μg, 48μg, and 72μg and placebo with about 30 patients per arm. Summary of sponsor's safety analysis is given below.

Study SC9921

Variable	Placebo (n=33)	RU-0211 24 μg (n=29)	RU-0211 48 μg (n=32)	RU-0211 72 μg (n=33)	P-value
At least 1 AE	13 (39.4%)	18 (62.1%)	24 (75%)	23 (69.7%)	0.0006
Relative Risk		1.6	1.9	1.8	
AE - GI	6 (18.2%)	10 (34.5%)	16 (50.0%)	16 (48.5%)	0.006
Relative Risk		1.9	2.7	2.7	
At least 1 Treatment-related AE	7 (21.2%)	10 (34.5%)	17 (53.1%)	20 (60.6%)	<0.001
Relative Risk		1.6	2.5	2.9	
Related AE - GI	4 (12.1%)	10 (34.5%)	14 (43.8%)	15 (45.5%)	0.004
Relative Risk		2.9	3.6	3.8	

Complied by this reviewer.
P-value was obtained by Cochran-Armitage test for trend.

As seen from table above, the number of patients experiencing AEs was dose-dependent, and the overall trend was statistically significant. The number of patients experiencing treatment related AEs was dose-dependent, and the overall trend was statistically significant. The number of patients experiencing gastrointestinal AEs was dose-dependent, and the overall trend was statistically significant. Furthermore, as seen from table above, the relative risk for low dose (24 μg) was smaller than middle dose (48 μg) for AE-GI, at least 1 treatment related AE, and related AE-GI (1.9 vs. 2.7, 1.6 vs. 2.5, 2.9 vs. 3.6, respectively).

The AE most commonly considered to be possibly, probably, or definitely related to study drug was nausea (30 patients, 23.6%).

The AE for which the frequency increased most dramatically with RU-0211 dose was nausea (0% of placebo patients, 17.2% of RU-0211 24 μg patients, 43.8% of RU-0211 48 μg patients, and 36.4% of RU-0211 72 μg patients).

The frequency of abdominal pain appeared to increase with RU-0211 dose.

The mean cumulative exposure and mean average daily exposure to suppositories were highest in the RU-0211 48 μg patients group (1.43 vs. 1.1 and 0.14 vs. 0.07, respectively).

For more details, see medical review.

4. FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

4.1 Gender, Race and Age

No conclusion on race can be drawn due to lack of representation of Black and other race. Similarly, there were very few patients aged 65 or older, no conclusion on age can be drawn.

Subgroup analyses were performed on the number of patients who were full responders or moderate responders for all 4 weeks by gender. In these analyses, patients with missing outcomes were set treatment failures. The results of subgroup analysis of the number of patients with treatment success are given below.

Number of Patients who were Full Responders or Moderate Responders

For All 4 Weeks by Subgroup

Reviewer's ITT Population with LOCF
Protocol SC0232

Gender	RU-0211 48μg	Placebo	Difference	95% C. I.
Male	10/15 (67%)	3/13 (23%)	44%	(10.5%, 76.7%)
Female	51/104 (49%)	33/105 (31%)	18%	(4.5%, 307%)

Gender	RU-0211 48μg	Placebo	Difference	95% C. I.
Male	6/13 (46%)	3/12 (25%)	21%	(-15.4%, 57.7%)
Female	48/107 (45%)	23/110 (21%)	24%	(11.8%, 36.1%)

As seen from tables above, treatment difference was statistically significant for females. The treatment difference for males failed to achieve statistical significance for Study SC0131 due to inadequate sample size.

4.2 Other Special/Subgroup Populations

No conclusion other special/subgroup population was drawn.

5. SUMMARY AND CONCLUSIONS

5.1 Statistical Issues and Collective Evidence

The sponsor has submitted two pivotal studies (SC0131 and SC0232) to support the claim.

For study SC0232, the sponsor's ITT analysis was not true ITT analysis. It did not include all randomized patients. It excluded more patients in RU-0211 48 μg group than in placebo group (8 vs. 1, p=0.0180, chi-square test). So, sponsor's ITT analysis may tend to be biased in favor of RU-0211 48 μg group.

Furthermore, in the sponsor's analysis of weekly response rate, it was also found that more patients in RU-0211 48 μg group were imputed by LOCF than in placebo at Weeks 3 and 4 (8 in RU-0211 48 μg group and 2 in placebo group). So, the sponsor's ITT analysis for weekly response rate may tend to be biased in favor of RU-0211 48 μg group.

As re-analysis for weekly response rate, this reviewer performed CMH (Cochran-Mantel-Haenszel) test using modified ridit scores for reviewer's ITT population without LOCF. In these analyses, patients with missing outcomes were set as no responders. Contrary to the sponsor's finding based on sponsor's ITT analysis with LOCF, it was found that treatment difference achieved statistical significance only at Week 1 (primary efficacy assessment time point) and Week 3 at significance level of 0.05 without adjustment for multiplicity.

For the more clinically meaningful efficacy parameter, where a responder is defined as a patient who had an SBM frequency rate of ≥ 3 per week for all 4 weeks, and who did not use rescue medication during or within 24 hours prior to the given week, and who did not drop out during the study due to lack of efficacy, this reviewer performed the responder analysis. In this analysis, patients with missing outcomes were set as no responders. This endpoint is more stringent than the pre-specified primary endpoint.

Both studies (SC0131 and SC0232) showed that for more stringent efficacy endpoint (≥ 3 SBMs/wk for all 4 weeks), the RU-0211 48 μg was superior to the placebo with treatment

differences of about 20% (41% for 48μg and 21% for placebo) and 16% (44% for 48μg and 28% for placebo) for studies SC0131 and SC0232, respectively.

In Study SC9921, a dose ranging Phase IIb study, the primary efficacy endpoint was pre-specified as number of SBMs. The primary time point was pre-specified as Week 3. There was slight imbalance in weekly average number of SBMs at baseline (1.1 for 24 μg, 1.3 to 1.4 for other groups). It failed to achieve statistical significance due to inadequate sample size. So, the efficacy analysis based on weekly average of SBM might be biased in favor of higher doses. It is more appropriate to asses the efficacy results based on the change from baseline.

This reviewer performed analysis of change of weekly average number of SBMs from baseline using Wilcoxon test. Results of this analysis showed that all doses were statistically significant from placebo at Week 1 and Week 2. But, they failed to achieve statistical significance at Week 3, pre-specified primary timepoint. No differences between the low dose (24 μg) and middle dose (48 μg) were observed at Week 2 and Week 3. At Week 1, middle dose (48 μg) was numerically slightly better than low dose (24 μg).

This reviewer performed post-hoc analyses for two stringent efficacy endpoints, the number of patients with increase of greater than or equal to one SBM/wk from baseline and number of patients with greater than or equal to 3 SBMs/wk. These efficacy endpoints were used for approval of Zelnorm. In the Zelnorm submission, CSBM/wk was assessed instead of SBM/wk.

The results of this reviewer's analyses for the number of patients with increase greater than or equal to one SBM/week and number of patients with greater than or equal to 3 SBMs/wk showed that all doses were numerically better than placebo for both two stringent endpoints and all timepoints (Week 1, Week 2, Week 3, and over the period Week 1 to Week 3). But, they failed to achieve statistical significance due to insufficient sample sizes. Furthermore, the low dose (24 μg) was close to the middle dose (48 μg) at Week 1, Week 3, and Week 1 to Week 3. However, the low dose (24 μg) was slightly better than the middle dose (48 μg) at Week 2. So, the minimum effective dose might be the low dose (24 μg). The low dose (24 μg) should be included in the Phase III studies.

5.2 Conclusions and Recommendations

The sponsor has submitted two pivotal studies (SC0131 and SC0232) to support the claim.

Study SC0131 showed that RU-0211 48 μg group was statistically significantly better than placebo group in terms of the primary efficacy endpoint, the spontaneous bowel movements (SBM) frequency rate during Week1 in subjects with constipation. The superiority was also shown for all secondary efficacy endpoints with exceptions for abdominal bloating and abdominal discomfort.

The efficacy results from study SC0131 were replicated in study SC0232 for the primary efficacy endpoint and for the most of secondary efficacy variables (SBM within 24 hours of first study drug, time to first SBM, degree of stool consistency, degree of straining, degree of

constipation and global assessment of treatment effectiveness, abdominal bloating and abdominal discomfort).

For study SC0232, the sponsor's ITT analysis was not true ITT analysis. It did not include all randomized patients. It excluded more patients in RU-0211 48 μg group than in placebo group (8 vs. 1, p=0.0180, chi-square test). So, sponsor's ITT analysis may tend to be biased in favor of RU-0211 48 μg group.

Furthermore, in the sponsor's analysis of weekly response rate, it was also found that more patients in RU-0211 48 μg group were imputed by LOCF than in placebo at Weeks 3 and 4 (8 in RU-0211 48 μg group and 2 in placebo group). So, the sponsor's ITT analysis for weekly response rate may tend to be biased in favor of RU-0211 48 μg group.

As re-analysis for weekly response rate, this reviewer performed CMH (Cochran-Mantel-Haenszel) test using modified ridit scores for reviewer's ITT population without LOCF. In these analyses, patients with missing outcomes were set as no responders. Contrary to the sponsor's finding based on sponsor's ITT analysis with LOCF, it was found that treatment difference achieved statistical significance only at Week 1 (primary efficacy assessment time point) and Week 3 at significance level of 0.05 without adjustment for multiplicity.

For the more clinically meaningful efficacy parameter, where a responder is defined as a patient who had an SBM frequency rate of ≥ 3 per week for all 4 weeks, and who did not use rescue medication during or within 24 hours prior to the given week, and who did not drop out during the study due to lack of efficacy, this reviewer performed the responder analysis. In this analysis, patients with missing outcomes were set as no responders. This endpoint is more stringent than the pre-specified primary endpoint.

Both studies (SC0131 and SC0232) showed that for more stringent efficacy endpoint (≥ 3 SBM/wk for all 4 weeks), the RU-0211 48 μg was superior to the placebo with treatment differences of about 20% and 16% for studies SC0131 and SC0232, respectively.

In Study SC9921, a dose ranging Phase IIb study, the primary efficacy endpoint was pre-specified as number of SBMs. The primary time point was pre-specified as Week 3. There was slight imbalance in weekly average number of SBMs at baseline (1.1 for 24 μg, 1.3 to 1.4 for other groups). It failed to achieve statistical significance due to inadequate sample size. So, the efficacy analysis based on weekly average of SBMs might be biased in favor of higher doses. It is more appropriate to asses the efficacy results based on the change from baseline.

This reviewer performed analysis of change of weekly average number of SBMs from baseline using Wilcoxon test. Results of this analysis showed that all doses were statistically significant from placebo at Week 1 and Week 2. But, they failed to achieve statistical significance at Week 3. No differences between the low dose (24 μg) and middle dose (48 μg) were observed at Week 2 and Week 3. At Week 1, middle dose (48 μg) was numerically slightly better than low dose (24 μg).

This reviewer performed post-hoc analyses for two stringent efficacy endpoints, the number of patients with increase of greater than or equal to one SBM/wk from baseline and number of patients with greater than or equal to 3 SBMs/wk. These efficacy endpoints were used for approval of Zelnorm. In the Zelnorm submission, CSBM/wk was assessed instead of SBM/wk.

The results of this reviewer's analyses for the number of patients with increase greater than or equal to one SBM/week and number of patients with greater than or equal to 3 SBMs/wk showed that all doses were numerically better than placebo for both two stringent endpoints and all timepoints (Week 1, Week 2, Week 3, and over the period Week 1 to Week 3). But, they failed to achieve statistical significance due to insufficient sample sizes. Furthermore, the low dose (24 μg) was close to the middle dose (48 μg) at Week 1, Week 3, and Week 1 to Week 3. However, the low dose (24 μg) was slightly better the middle dose (48 μg) at Week 2. So, the minimum effective dose might be the low dose (24 μg). The low dose (24 μg) should be included in the Phase III studies.

In conclusion, both studies (SC0131 and SC0232) showed that the RU-0211 48 μg was superior to the placebo for pre-specified primary efficacy endpoint and most secondary efficacy endpoints. Even for more stringent efficacy endpoint (≥ 3 SBMs/wk for all 4 weeks), the results from reviewer's post-hoc analysis revealed that the RU-0211 48 μg was superior to the placebo with treatment differences of about 20% and 16% for studies SC0131 and SC0232, respectively. However, the results form reviewer's post hoc analysis for Study SC9921 revealed that the RU-0211 48 μg might not be the minimum effective dose.

6. APPENDIX

Table 1 Summary of Demographic and Baseline Characteristics --- Protocol SC0232

Characteristics	Placebo (N=118)	(ITT Subjects) RU-0211 48 µg (N=119)	Between Treatment p-value
Sex			0.8409
Male	13 (11.0%)	15 (12.6%)	
Female	105 (89.0%)	104 (87.4%)	
Race			0.6933
White	89 (75.4%)	90 (75.6%)	
Black	12 (10.2%)	13 (10.9%)	
Asian	1 (0.8%)	4 (3.4%)	
Hispanic	14 (11.9%)	11 (9.2%)	
Other Races	2 (1.7%)	1 (0.8%)	
Age (months)			0.6560
Mean (SD)	45.4 (13.2)	46.2 (12.1)	
Age			1.000
18 to 64	108 (91.5%)	109 (91.6%)	
≥ 65	10 (8.5%)	10 (8.4%)	
Height (in)			0.1548
Mean (SD)	64.4 (3.42)	65.1 (3.75)	
Weight (lb)			0.5696
Mean (SD)	157.1 (35.8)	159.7 (35.9)	
Flexible Sigmoidoscopy			0.2580
No	91 (77.1%),	99 (83.2%)	
Yes	27 (22.9%)	20 (16.8%)	
Barium Enema			1.0000
No	115 (97.5%)	116 (97.5%)	
Yes	3 (2.5%)	3 (2.5%)	
Colonoscopy			0.4120
No	25 (21.2%)	20 (16.8%)	
Yes	93 (78.8%)	99 (83.2%)	

Copied from Table 11-1.
P-values are based on t-tests for age and height.
P-values are based on Fisher's Exact tests for categorical variables and binary variables.

Table 1 Summary of Demographic and Baseline Characteristics --- Protocol SC0232 (Continued)

Characteristics	Placebo (N=118)	((ITT Subjects) RU-0211 48 µg (N=119)	Between Treatment p-value
Irritable Bowel Syndrome			
No	98 (83.1%)	106 (89.1%)	0.1942
Yes	20 (16.9%)	13 (10.9%)	
Gastroesophogeal Reflux Disease			
No	84 (71.2%)	85 (71.4%)	1.0000
Yes	34 (28.8%)	34 (28.6%)	

Copied from Table 11-1.
P-values are based on t-tests for age and height.
P-values are based on Fisher's Exact tests for categorical variables and binary variables.

Table 2 Overall Summaries of SBM Frequency Rates [1] --- Protocol SC0232

Week Treatment Group Statistic	ITT Subjects with LOCF	ITT Subjects without LOCF	ITT Subjects Who Completed the Study	Per Protocol Subjects
Baseline				
Placebo, n	118	118	107	117
Mean (Std Dev)	1.52 (0.801)	1.52 (0.801)	1.51 (0.793)	1.53 (0.792)
Median	1.5	1.5	1.5	1.5
RU-0211 48 µg, n	119	119	98	118
Mean (Std Dev)	1.28 (0.881)	1.28 (0.881)	1.26 (0.884)	1.28 (0.881)
Median	1.5	1.5	1.3	1.5
p-value [2]	0.0126	0.0126	0.0084	0.0089
Week 1				
Placebo, n	116	116	107	106
Mean (Std Dev)	3.99 (2.706)	3.99 (2.706)	4.05 (2.661)	4.05 (2.684)
Median	3.5	3.5	4.0	4.0
RU-0211 48 µg, n	111	111	99	98
Mean (Std Dev)	5.89 (4.022)	5.89 (4.022)	5.78 (3.669)	6.07 (4.059)
Median	5.0	5.0	5.0	5.0
p-value [2]	<0.0001	<0.0001	0.0003	0.0002
Overall p-value [3]	<0.0001	<0.0001	<0.0001	<0.0001
Week 2				
Placebo, n	116	114	107	107
Mean (Std Dev)	3.55 (2.670)	3.56 (2.659)	3.65 (2.678)	3.68 (2.676)
Median	3.0	3.0	3.0	3.0
RU-0211 48 µg, n	111	110	99	98
Mean (Std Dev)	4.96 (4.208)	4.98 (4.224)	4.93 (4.003)	4.97 (4.028)
Median	4.0	4.0	4.0	4.0
p-value [2]	0.0487	0.0559	0.1227	0.1219
Week 3				
Placebo, n	116	112	107	106
Mean (Std Dev)	3.36 (2.755)	3.38 (2.767)	3.48 (2.774)	3.45 (2.781)
Median	3.0	3.0	3.0	3.0
RU-0211 48 µg, n	111	103	99	95
Mean (Std Dev)	5.56 (4.560)	5.54 (4.428)	5.60 (4.415)	5.55 (4.400)
Median	5.0	5.0	5.0	5.0
p-value [2]	0.0004	0.0011	0.0014	0.0012
Week 4				
Placebo, n	116	106	105	104
Mean (Std Dev)	3.46 (2.861)	3.61 (2.881)	3.63 (2.889)	3.62 (2.900)
Median	3.0	3.0	3.0	3.0
RU-0211 48 µg, n	111	99	99	89
Mean (Std Dev)	5.37 (4.804)	5.39 (4.698)	5.39 (4.698)	5.41 (4.618)
Median	4.3	4.1	4.1	4.1
p-value [2]	0.0068	0.0368	0.0411	0.0246

[1] SBM Frequency Rate: (Number of SBMs/Number of days) x 7.
[2] P-values are based on van Elteren tests adjusted for pooled center.
[3] Overall p-value is based on the final mixed model testing for overall treatment effect.

Table 3 Overall Summaries of BM Frequency Rates [1] --- Protocol SC0232

Week Treatment Group Statistic	ITT Subjects with LOCF	ITT Subjects without LOCF	ITT Subjects Who Completed the Study	Per Protocol Subjects
Baseline				
Placebo, n	118	118	107	117
Mean (Std Dev)	2.23 (1.135)	2.23 (1.135)	2.24 (1.173)	2.23 (1.140)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	118	118	98	118
Mean (Std Dev)	2.09 (1.095)	2.09 (1.095)	2.11 (1.113)	2.09 (1.095)
Median	2.0	2.0	2.0	2.0
p-value [2]	0.2397	0.2397	0.3330	0.2620
Week 1				
Placebo, n	116	116	107	106
Mean (Std Dev)	4.09 (2.669)	4.09 (2.669)	4.08 (2.667)	4.05 (2.684)
Median	4.0	4.0	4.0	4.0
RU-0211 48 µg, n	111	111	99	98
Mean (Std Dev)	5.99 (3.956)	5.99 (3.956)	5.82 (3.638)	6.07 (4.059)
Median	5.0	5.0	5.0	5.0
p-value [2]	<0.0001	<0.0001	0.0003	0.0002
Overall p-value [3]	<0.0001	<0.0001	0.0001	<0.0001
Week 2				
Placebo, n	116	114	107	107
Mean (Std Dev)	4.00 (2.402)	3.99 (2.409)	4.04 (2.441)	4.09 (2.410)
Median	4.0	4.0	4.0	4.0
RU-0211 48 µg, n	111	110	99	95
Mean (Std Dev)	5.32 (4.054)	5.34 (4.068)	5.31 (3.846)	5.35 (3.872)
Median	4.0	4.0	4.0	4.0
p-value [2]	0.0786	0.0838	0.1037	0.1542
Week 3				
Placebo, n	116	112	107	106
Mean (Std Dev)	3.99 (2.637)	4.01 (2.662)	4.06 (2.626)	4.03 (2.639)
Median	3.1	3.3	4.0	3.5
RU-0211 48 µg, n	111	100	99	95
Mean (Std Dev)	5.92 (4.419)	5.94 (4.266)	6.00 (4.245)	5.87 (4.198)
Median	5.0	5.0	5.0	5.0
p-value [2]	0.0037	0.0089	0.0052	0.0101
Week 4				
Placebo, n	116	106	105	104
Mean (Std Dev)	3.92 (2.691)	3.99 (2.702)	4.01 (2.706)	4.00 (2.717)
Median	3.2	4.0	4.0	4.0
RU-0211 48 µg, n	111	99	99	89
Mean (Std Dev)	5.65 (4.628)	5.70 (4.492)	5.70 (4.492)	5.71 (4.438)
Median	5.0	4.7	4.7	4.7
p-value [2]	0.0105	0.0253	0.0287	0.0219

[1] BM Frequency Rate: (Number of BMs/Number of days) x 7.
[2] P-values are based on van Elteren tests adjusted for pooled center.
[3] Overall p-value is based on the final mixed model testing for overall treatment effect.

Table 4 Summary of Weekly Responder Status --- Protocol SC0232

Week Treatment Group Responder Status	ITT Subjects with LOCF	ITT subjects without LOCF	ITT subjects who completed the study	Per Protocol Subjects
Week 1				
Placebo , n	118	118	107	118
Full Responder	57/117 (48.7%)	57/117 (48.7%)	54/107 (50.0%)	53/106 (50.0%)
Moderate Responder	14/117 (12.0%)	14/117 (12.0%)	14/107 (13.1%)	13/106 (12.3%)
Non-Responder	46/117 (39.3%)	46/117 (39.3%)	39/107 (36.4%)	40/106 (37.3%)
RU-0211 48 µg, n	119	119	99	119
Full Responder	80/111 (72.1%)	80/111 (72.1%)	71/99 (71.7%)	72/98 (73.5%)
Moderate Responder	16/111 (14.4%)	16/111 (14.4%)	15/99 (15.2%)	15/98 (15.3%)
Non-Responder	15/111 (13.5%)	15/111 (13.5%)	13/99 (13.1%)	11/98 (11.2%)
P-value	<0.0001	<0.0001	0.0004	0.0001
Week 2				
Placebo , n	118	118	107	118
Full Responder	50/117 (42.7%)	50/114 (43.9%)	48/107 (44.9%)	49/107 (45.8%)
Moderate Responder	13/117 (11.1%)	13/114 (1141%)	12/107 (11.2%)	12/107 (11.2%)
Non-Responder	54/117 (46.2%)	51/114 (44.7%)	47/107 (43.9%)	46/107 (43.0%)
RU-0211 48 µg, n	119	119	99	119
Full Responder	64/111 (57.7%)	64/110 (58.2%)	57/99 (57.6%)	55/95 (57.9%)
Moderate Responder	13/111 (11.7%)	12/110 (10.9%)	12/99 (12.1%)	11/95 (11.6%)
Non-Responder	34/111 (30.6%)	34/110 (30.9%)	30/99 (30.3%)	29/95 (30.5%)
P-value	0.0171	0.0303	0.0588	0.0808
Week 3				
Placebo , n	118	118	107	118
Full Responder	42/117 (35.9%)	42/112 (37.5%)	42/107 (39.3%)	41/106 (38.7%)
Moderate Responder	15/117 (12.8%)	14/112 (12.5%)	13/107 (12.1%)	13/106 (12.3%)
Non-Responder	60/117 (51.3%)	56/112 (50.0%)	52/107 (48.6%)	52/106 (49.1%)
RU-0211 48 µg, n	119	118	99	119
Full Responder	68/111 (61.3%)	61/100 (61.0%)	61/99 (61.6%)	59/95 (62.1%)
Moderate Responder	11/111 (9.9%)	10/100 (10.0%)	10/99 (10.1%)	10/95 (10.5%)
Non-Responder	32/111 (28.8%)	29/100 (29.0%)	28/99 (28.3%)	26/95 (27.4%)
P-value	0.0002	0.0012	0.0025	0.0018
Week 4				
Placebo , n	118	118	107	118
Full Responder	45/117 (38.5%)	45/106 (42.5%)	45/105 (42.9%)	44/104 (42.3%)
Moderate Responder	17/117 (14.5%)	15/106 (14.2%)	14/105 (14.3%)	15/104 (14.4%)
Non-Responder	55/117 (47.0%)	46/106 (43.4%)	45/105 (42.9%)	45/104 (43.3%)
RU-0211 48 µg, n	119	119	99	119
Full Responder	66/111 (59.5%)	59/99 (59.6%)	59/99 (59.6%)	54/89 (60.7%)
Moderate Responder	13/111 (11.7%)	12/99 (12.1%)	12/99 (12.1%)	11/89 (12.4%)
Non-Responder	32 /111 (28.8%)	28/99 (28.3%)	28/99 (28.3%)	24/89 (27.0%)
P-value	0.0022	0.0277	0.0324	0.0227

Copied from Tables 14.2.4.1, 14.2.4.2, 14.2.4.3, and 14.2.4.4.
p-values are based on van Elteren tests adjusted for pooled center.

Table 5 Summary of Subjects with SBMs within 24 Hours after the First Study Drug Administration --- Protocol SC0232

SBMs Within 24 Hours

(Intent-to-Treat Subjects without LOCF)

Treatment	SBM within 24 hours	p-value
Placebo	37/118 (31.4%)	<0.0001
RU-0211 48 µg	73/119 (61.3%)	

Copied from Table 14.2.5.1

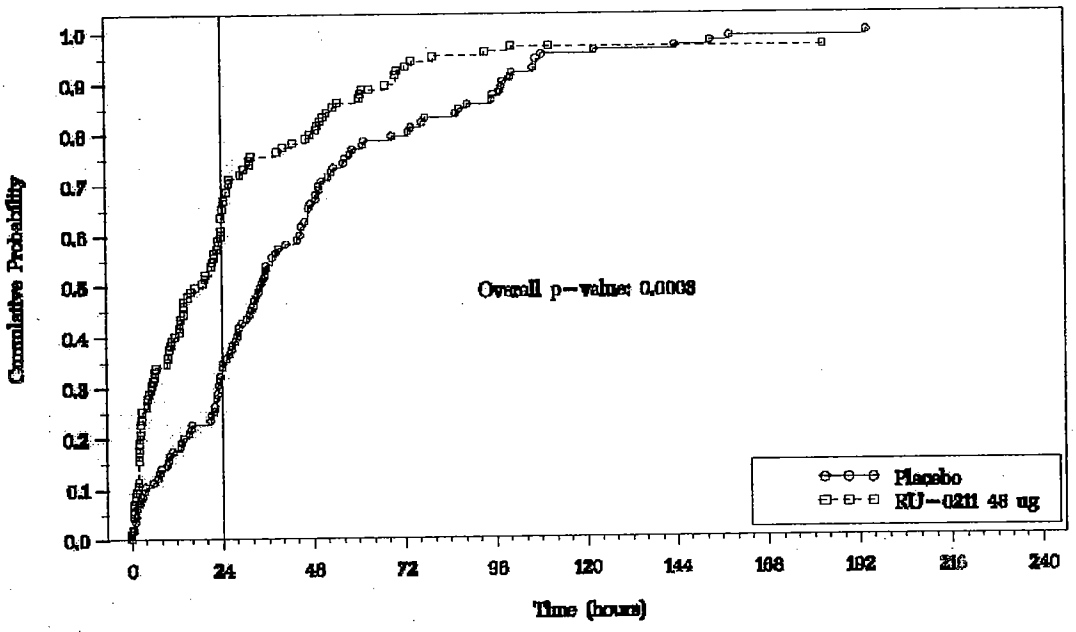
(Per Protocol Subjects)

Treatment	SBM within 24 hours	p-value
Placebo	45/118 (28.8%)	0.0002
RU-0211 48 µg	60/119 (50.4%)	

Copied from Table 14.2.5.2

APPEARS THIS WAY
ON ORIGINAL

Figure 6 Kaplan-Meier Curve for Time to First SBM (ITT subjects with LOCF) --- Protocol SC0232



APPEARS THIS WAY
ON ORIGINAL

Table 7 Overall Summary of Average Stool Consistency [1] --- Protocol SC0232

Week Treatment Group Statistic	ITT Subjects with LOCF	ITT Subjects without LOCF	ITT Subjects Who Completed the Study	Per Protocol Subjects
Baseline				
Placebo, n	110	110	100	110
Mean (Std Dev)	2.78 (0.770)	2.78 (0.770)	2.80 (0.769)	2.78 (0.770)
Median	3.0	3.0	3.0	3.0
RU-0211 48 µg, n	96	96	79	96
Mean (Std Dev)	2.69 (0.834)	2.69 (0.834)	2.74 (0.850)	2.69 (0.834)
Median	2.9	2.9	3.0	2.9
p-value [2]	0.9854	0.9854	0.7386	0.9854
Week 1				
Placebo, n	113	113	106	105
Mean (Std Dev)	2.55 (0.759)	2.55 (0.759)	2.54 (0.774)	2.54 (0.741)
Median	2.5	2.5	2.5	2.5
RU-0211 48 µg, n	111	111	95	97
Mean (Std Dev)	1.75 (0.891)	1.75 (0.891)	1.85 (0.867)	1.77 (0.[illegible])
Median	1.8	1.8	2.0	1.8
p-value [2]	<0.0001	<0.0001	<0.0001	<0.0001
Week 2				
Placebo, n	114	100	96	96
Mean (Std Dev)	2.50 (0.788)	2.46 (0.772)	2.46 (0.784)	2.46 (0.778)
Median	2.4	2.3	2.3	2.3
RU-0211 48 µg, n	113	94	90	87
Mean (Std Dev)	1.83 (0.853)	1.91 (0.793)	1.93 (0.796)	1.93 (0.799)
Median	2.0	2.0	2.0	2.0
p-value [2]	<0.0001	<0.0001	<0.0001	<0.0001
Week 3				
Placebo, n	114	97	94	93
Mean (Std Dev)	2.49 (0.762)	2.41 (0.707)	2.39 (0.710)	2.40 (0.716)
Median	2.3	2.3	2.3	2.3
RU-0211 48 µg, n	114	91	91	88
Mean (Std Dev)	1.74 (0.860)	1.86 (0.828)	1.86 (0.828)	1.87 (0.836)
Median	1.8	2.0	2.0	2.0
p-value [2]	<0.0001	<0.0001	<0.0001	<0.0001
Week 4				
Placebo, n	114	93	93	92
Mean (Std Dev)	2.45 (0.754)	2.39 (0.734)	2.39 (0.734)	2.39 (0.738)
Median	2.3	2.3	2.3	2.3
RU-0211 48 µg, n	114	87	87	83
Mean (Std Dev)	1.74 (0.814)	1.90 (0.780)	1.90 (0.780)	1.93 (0.775)
Median	2.0	2.0	2.0	2.0
p-value [2]	<0.0001	0.0017	0.0017	0.0059

[1] Stool consistency: 0(Very loose), 1(Loose), 2(Normal), 3(Hard), and 4(Very hard).
[2] P-values are based on van Elteren tests adjusted for pooled center.

Table 8 Overall Summary of Average Degree of Straining [1] --- Protocol SC0232

Week Treatment Group Statistic	ITT Subjects with LOCF	ITT Subjects without LOCF	ITT Subjects Who Completed the Study	Per Protocol Subjects
Baseline				
Placebo, n	109	109	99	109
Mean (Std Dev)	2.36 (0.925)	2.36 (0.925)	2.41 (0.924)	2.36 (0.925)
Median	2.4	2.4	2.5	2.4
RU-0211 48 µg, n	95	95	78	95
Mean (Std Dev)	2.34 (0.942)	2.34 (0.942)	2.42 (0.992)	2.34 (0.942)
Median	2.3	2.3	2.4	2.3
p-value [2]	0.7985	0.7985	0.7373	0.7985
Week 1				
Placebo, n	112	112	106	104
Mean (Std Dev)	1.96 (0.946)	1.96 (0.946)	2.00 (0.941)	1.97 (0.951)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	109	109	94	
Mean (Std Dev)	1.48 (0.946)	1.48 (0.962)	1.53 (0.966)	
Median	2.0	1.6	1.7	1.6
p-value [2]	0.0017	0.0017	0.0082	0.0023
Week 2				
Placebo, n	114	100	96	96
Mean (Std Dev)	1.91 (0.974)	1.94 (0.938)	1.95 (0.951)	1.93 (0.946)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	112	94	90	87
Mean (Std Dev)	1.43 (0.840)	1.48 (0.799)	1.50 (0.804)	1.50 (0.816)
Median	1.5	1.5	1.5	1.5
p-value [2]	0.0003	0.0023	0.0066	0.0071
Week 3				
Placebo, n	114	97	94	93
Mean (Std Dev)	1.81 (0.990)	1.79 (0.982)	1.76 (0.959)	1.79 (0.966)
Median	1.8	1.8	1.8	1.8
RU-0211 48 µg, n	113	91	91	88
Mean (Std Dev)	1.36 (0.834)	1.48 (0.824)	1.48 (0.824)	1.43 (0.835)
Median	1.4	1.5	1.5	1.6
p-value [2]	0.0018	0.0529	0.0673	0.0463
Week 4				
Placebo, n	114	92	92	91
Mean (Std Dev)	1.79 (0.978)	1.79 (0.962)	1.79 (0.962)	1.81 (0.956)
Median	2.0	1.9	1.9	2.0
RU-0211 48 µg, n	113	86	86	82
Mean (Std Dev)	1.33 (0.827)	1.45 (0.821)	1.45 (0.821)	1.45 (0.836)
Median	1.4	1.5	1.5	1.5
p-value [2]	0.0002	0.0182	0.0182	0.0270

[1] Straining: 0(Absent), 1(Mild), 2(Moderate), 3(Severe), and 4(Very severe).
[2] P-values are based on van Elteren tests adjusted for pooled center.

Table 9 Overall Summary of Average Severity of Constipation [1] --- Protocol SC0232

Week Treatment Group Statistic	ITT Subjects with LOCF	ITT Subjects without LOCF	ITT Subjects Who Completed the Study	Per Protocol Subjects
Baseline				
Placebo, n	117	117	106	116
Mean (Std Dev)	2.99 (0.760)	2.99 (0.760)	3.01 (0.737)	2.99 (0.763)
Median	3.0	3.0	3.0	3.0
RU-0211 48 µg, n	119	119	99	119
Mean (Std Dev)	3.00 (0.823)	3.00 (0.823)	3.04 (0.820)	3.00 (0.823)
Median	3.0	3.0	3.0	3.0
p-value [2]	0.7766	0.7766	0.5272	0.7765
Week 1				
Placebo, n	111	111	103	101
Mean (Std Dev)	2.31 (1.068)	2.31 (1.068)	2.28 (1.033)	2.30 (1.082)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	107	106	94	92
Mean (Std Dev)	1.84 (1.253)	1.85 (1.256)	1.82 (1.218)	[illegible]
Median	2.0	2.0	2.0	2.0
p-value [2]	0.0061	0.0075	0.0083	0.0007
Week 2				
Placebo, n	115	98	94	91
Mean (Std Dev)	1.99 (1.047)	1.97 (1.010)	1.95 (0.977)	2.01 (0.972)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	115	97	88	85
Mean (Std Dev)	1.64 (1.141)	1.62 (1.084)	1.63 (1.032)	1.64 (1.067)
Median	2.0	2.0	2.0	2.0
p-value [2]	0.0243	0.0264	0.0544	0.0184
Week 3				
Placebo, n	116	105	101	103
Mean (Std Dev)	2.14 (1.046)	2.13 (1.029)	2.10 (1.025)	2.13 (1.016)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	116	94	93	90
Mean (Std Dev)	1.78 (1.143)	1.76 (1.094)	1.74 (1.092)	1.76 (1.115)
Median	2.0	2.0	2.0	2.0
p-value [2]	0.0265	0.0365	0.0391	0.0426
Week 4				
Placebo, n	116	92	91	91
Mean (Std Dev)	2.22 (1.133)	2.21 (1.084)	2.20 (1.086)	2.22 (1.084)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	116	91	91	84
Mean (Std Dev)	1.71 (1.202)	1.74 (1.124)	1.74 (1.124)	1.73 (1.144)
Median	2.0	2.0	2.0	2.0
p-value [2]	0.0022	0.0241	0.0319	0.0229

[1] Severity of constipation: 0(Absent), 1(Mild), 2(Moderate), 3(Severe), and 4(Very severe).
[2] P-values are based on van Elteren tests adjusted for pooled center.

Table 10 Overall Summary of Global Assessment of Treatment Effectiveness [1] --- Protocol SC0232

Week Treatment Group Statistic	ITT Subjects with LOCF	ITT Subjects without LOCF	ITT Subjects Who Completed the Study	Per Protocol Subjects
Week 1				
Placebo, n	111	111	103	101
Mean (Std Dev)	1.22 (1.186)	1.22 (1.186)	1.24 (1.184)	1.20 (1.158)
Median	1.0	1.0	1.0	1.0
RU-0211 48 µg, n	107	106	94	92
Mean (Std Dev)	1.88 (1.186)	1.87 (1.265)	1.89 (1.222)	1.99 (1.227)
Median	1.0	2.0	2.0	2.0
p-value [2]	<0.0001	<0.0001	0.0002	<0.0001
Week 2				
Placebo, n	115	98	94	91
Mean (Std Dev)	1.22 (1.153)	1.20 (1.148)	1.22 (1.137)	1.20 (1.157)
Median	1.0	1.0	1.0	
RU-0211 48 µg, n	115	97	88	84
Mean (Std Dev)	1.95 (1.323)	1.93 (1.277)	1.89 (1.245)	1.85 (1.268)
Median	2.0	2.0	2.0	2.0
p-value [2]	<0.0001	<0.0001	0.0002	0.0007
Week 3				
Placebo, n	116	105	101	103
Mean (Std Dev)	1.17 (1.189)	1.16 (1.153)	1.15 (1.126)	1.13 (1.126)
Median	1.0	1.0	1.0	1.0
RU-0211 48 µg, n	116	94	93	90
Mean (Std Dev)	1.86 (1.318)	1.84 (1.298)	1.85 (1.302)	1.86 (1.320)
Median	2.0	2.0	2.0	2.0
p-value [2]	0.0001	0.0005	0.0004	0.0002
Week 4				
Placebo, n	116	92	91	91
Mean (Std Dev)	1.22 (1.277)	1.25 (1.237)	1.26 (1.237)	1.24 (1.241)
Median	1.0	1.0	1.0	1.0
RU-0211 48 µg, n	116	91	91	84
Mean (Std Dev)	1.97 (1.328)	1.95 (1.294)	1.95 (1.294)	1.99 (1.313)
Median	2.0	2.0	2.0	2.0
p-value [2]	<0.0001	0.0368	0.0006	0.0003
Follow-up				
Placebo, n	109	109	107	109
Mean (Std Dev)	1.44 (1.343)	1.44 (1.343)	1.44 (1.340)	1.44 (1.343)
Median	1.0	1.0	1.0	1.0
RU-0211 48 µg, n	102	102	99	102
Mean (Std Dev)	2.14 (1.483)	2.14 (1.483)	2.14 (1.478)	2.14 (1.483)
Median	2.0	2.0	2.0	2.0
p-value [2]	0.0004	0.0004	0.0007	0.0004

[1] Treatment effectiveness: 0(Not at all effective), 1(A little bit effective), 2(Moderately effective), 3(Quite a bit effective), and 4(Very effective).
[2] P-values are based on van Elteren tests adjusted for pooled center.

Table 11 Overall Summary of Abdominal Bloating [1] --- Protocol SC0232

Week Treatment Group Statistic	ITT Subjects with LOCF	ITT Subjects without LOCF	ITT Subjects Who Completed the Study	Per Protocol Subjects
Baseline				
Placebo, n	118	118	107	117
Mean (Std Dev)	2.18 (0.912)	2.18 (0.912)	2.12 (0.918)	2.18 (0.916)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	119	119	99	115
Mean (Std Dev)	2.25 (1.027)	2.25 (1.027)	2.25 (1.043)	2.25 (1.027)
Median	2.0	2.0	2.0	2.0
p-value [2]	0.4985	0.4985	0.2533	0.5271
Week 1				
Placebo, n	111	111	103	101
Mean (Std Dev)	1.71 (1.107)	1.71 (1.107)	1.67 (1.088)	1.70 (1.091)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	107	106	94	92
Mean (Std Dev)	1.44 (1.126)	1.44 (1.130)	1.39 (1.109)	1.36 (1.078)
Median	1.0	1.0	1.0	1.0
p-value [2]	0.0380	0.0439	0.0701	0.0126
Week 2				
Placebo, n	115	98	94	91
Mean (Std Dev)	1.49 (1.079)	1.37 (1.078)	1.38 (1.079)	1.42 (1.086)
Median	1.0	1.0	1.0	1.0
RU-0211 48 µg, n	115	97	88	85
Mean (Std Dev)	1.41 (1.050)	1.40 (1.027)	1.38 (1.009)	1.42 (1.004)
Median	1.0	1.0	1.0	1.0
p-value [2]	0.6274	0.9012	0.9142	0.7485
Week 3				
Placebo, n	116	105	101	103
Mean (Std Dev)	1.71 (1.039)	1.71 (1.063)	1.70 (1.044)	1.70 (1.046)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	116	94	93	90
Mean (Std Dev)	1.50 (1.009)	1.47 (0.969)	1.46 (0973)	1.46 (0.985)
Median	2.0	1.5	1.0	1.0
p-value [2]	0.1788	0.2011	0.1905	0.1955
Week 4				
Placebo, n	116	92	91	91
Mean (Std Dev)	1.59 (1.096)	1.48 (1.084)	1.47 (1.089)	1.49 (1.079)
Median	1.5	1.0	1.0	1.0
RU-0211 48 µg, n	116	91	91	89
Mean (Std Dev)	1.39 (1.053)	1.34 (1.013)	1.34 (1.013)	1.35 (1.012)
Median	1.0	1.0	1.0	1.0
p-value [2]	0.2800	0.5897	0.6503	0.4990

[1] Bloating: 0(Absent), 1(Mild), 2(Moderate), 3(Severe), and 4(Very severe).
[2] P-values are based on van Elteren tests adjusted for pooled center.

Table 12 Overall Summary of Abdominal Discomfort [1] --- Protocol SC0232

Week Treatment Group Statistic	ITT Subjects with LOCF	ITT Subjects without LOCF	ITT Subjects Who Completed the Study	Per Protocol Subjects
Baseline				
Placebo, n	118	118	107	117
Mean (Std Dev)	1.84 (0.915)	1.84 (0.915)	1.83 (0.916)	1.84 (0.919)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	119	119	99	119
Mean (Std Dev)	1.88 (1.018)	1.88 (1.018)	1.88 (1.072)	1.88 (1.018)
Median	2.0	2.0	2.0	2.0
p-value [2]	0.8589	0.8589	0.9636	0.8708
Week 1				
Placebo, n	111	111	103	101
Mean (Std Dev)	1.40 (1.047)	1.40 (1.047)	1.42 (1.053)	1.39 (1.029)
Median	1.0	1.0	1.0	1.0
RU-0211 48 µg, n	107	106	94	92
Mean (Std Dev)	1.23 (0.967)	1.25 (0.964)	1.16 (0.942)	1.14 (0.872)
Median	1.0	1.0	1.0	1.0
p-value [2]	0.1514	0.1844	0.0986	0.0680
Week 2				
Placebo, n	115	98	94	91
Mean (Std Dev)	1.14 (1.042)	1.09 (1.026)	1.12 (1.025)	1.14 (1.039)
Median	1.0	1.0	1.0	1.0
RU-0211 48 µg, n	115	97	88	85
Mean (Std Dev)	1.16 (1.056)	1.15 (1.054)	1.11 (1.055)	1.13 (1.067)
Median	1.0	1.0	1.0	1.0
p-value [2]	0.8716	0.8796	0.8465	0.7559
Week 3				
Placebo, n	116	105	101	103
Mean (Std Dev)	1.41 (1.120)	1.46 (1.127)	1.45 (1.109)	1.44 (1.109)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	116	94	93	90
Mean (Std Dev)	1.34 (0.997)	1.31 (0.973)	1.30 (0.976)	1.30 (0.977)
Median	1.0	1.0	1.0	1.0
p-value [2]	0.8060	0.5554	0.6047	0.5948
Week 4				
Placebo, n	116	92	91	91
Mean (Std Dev)	1.47 (1.168)	1.37 (1.146)	1.36 (1.150)	1.38 (1.143)
Median	1.0	1.0	1.0	1.0
RU-0211 48 µg, n	116	91	91	84
Mean (Std Dev)	1.24 (1.018)	1.16 (0.969)	1.16 (0.969)	1.15 (0.976)
Median	1.0	1.0	1.0	1.0
p-value [2]	0.1393	0.3827	0.4362	0.2562

[1] Bloating: 0(Absent), 1(Mild), 2(Moderate), 3(Severe), and 4(Very severe).
[2] P-values are based on van Elteren tests adjusted for pooled center.

Table 13 Summary of Demographic and Baseline Characteristics --- Protocol SC0131

Characteristics	Placebo (N=122)	(ITT Subjects) RU-0211 48 µg (N=120)	Between Treatment p-value
Sex			0.7988
Male	12 (9.8%)	13 (10.8%)	
Female	110 (90.2%)	107 (89.2%)	
Race			0.4884
White	103 (84.4%)	105 (87.5%)	
Black	12 (9.8%)	9 (7.5%)	
Asian	2 (1.6%)	0 (0.0%)	
Hispanic	5 (4.1%)	5 (4.2%)	
Other Races	0 (0.0%)	1 (0.8%)	
Age (months)			0.5086
Mean (SD)	49.1 (12.9)	48.0 (12.3)	
Age			0.3435
18 to 64	103 (84.4%)	107 (89.2%)	
≥ 65	19 (15.6%)	13 (10.8%)	
Height (in)			0.6998
Mean (SD)	65.2 (3.43)	65.0 (3.18)	
Flexible Sigmoidoscopy			0.8218
No	85 (69.7%),	82 (68.3%)	
Yes	37 (30.3%)	38 (31.7%)	
Barium Enema			0.6684
No	111 (91.0%)	111 (92.5%)	
Yes	11 (9.0%)	9 (7.5%)	
Colonoscopy			0.6138
No	35 (28.7%)	38 (31.7%)	
Yes	87 (71.3%)	82 (68.3%)	

Copied from Table 11-1.
P-values are based on t-tests for age and height.
P-values are based on Chi-square tests for categorical variables and binary variables.

Table 13 Summary of Demographic and Baseline Characteristics --- Protocol SC0131 (Continued)

Characteristics	Placebo (N=122)	((ITT Subjects) RU-0211 48 µg (N=120)	Between Treatment p-value
Irritable Bowel Syndrome			
No	96 (78.7%)	88 (73.3%)	0.3292
Yes	26 (21.3%)	32 (26.7%)	
Gastroesophogeal Reflux Disease			
No	88 (72.1%)	84 (70.0%)	0.7147
Yes	34 (27.9%)	36 (30.0%)	

Copied from Table 11-1.
P-values are based on t-tests for age and height.
P-values are based on Fisher's Exact tests for categorical variables and binary variables.

Table 14 Overall Summaries of SBM Frequency Rates [1] --- Protocol SC0131

Week Treatment Group Statistic	ITT Subjects with LOCF	ITT subjects without LOCF	ITT subjects who completed the study	Per Protocol Subjects
Baseline				
Placebo , n	119	119	115	116
Mean (Std Dev)	1.47 (1.325)	1.47 (1.325)	1.47 (1.344)	1.37 (0.854)
Median	1.5	1.5	1.5	1.5
RU-0211 48 µg, n	120	120	106	111
Mean (Std Dev)	1.37 (0.873)	1.37 (0.873)	1.34 (0.853)	1.35 (0.845)
Median	1.5	1.5	1.5	1.5
P-value	0.6120	0.6120	0.4856	0.5881
Week 1				
Placebo , n	122	122	118	100
Mean (Std Dev)	3.46 (2.285)	3.46 (2.285)	3.49 (2.308)	3.65 (2.062)
Median	3.0	3.0	3.0	3.0
RU-0211 48 µg, n	116	116	105	101
Mean (Std Dev)	5.69 (4.417)	5.69 (4.417)	5.63 (4.432)	6.03 (4.378)
Median	5.0	5.0	5.0	5.0
P-value	0.0001	0.0001	0.0002	<0.0001
Overall p-value	<0.0001	<0.0001	<0.0001	<0.0001
Week 2				
Placebo , n	122	120	118	115
Mean (Std Dev)	3.18 (2.530)	3.20 (2.544)	3.20 (2.561)	3.18 (2.502)
Median	3.0	3.0	3.0	3.0
RU-0211 48 µg, n	116	114	105	106
Mean (Std Dev)	5.06 (4.076)	5.04 (4.072)	5.06 (3.957)	5.13 (4.096)
Median	4.0	4.0	4.0	4.0
P-value	0.0017	0.0024	0.0008	0.0016
Week 3				
Placebo , n	122	119	118	115
Mean (Std Dev)	2.84 (2.231)	2.87 (2.251)	2.88 (2.254)	2.83 (2.194)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	116	108	104	102
Mean (Std Dev)	5.25 (4.875)	5.37 (4.939)	5.22 (4.308)	5.51 (5.019)
Median	5.0	5.0	5.0	5.0
P-value	0.0002	<0.0001	<0.0001	<0.0001
Week 4				
Placebo , n	122	118	117	111
Mean (Std Dev)	2.91 (2.357)	2.96 (2.373)	2.98 (2.376)	3.02 (2.348)
Median	2.3	2.3	2.4	3.0
RU-0211 48 µg, n	116	104	104	103
Mean (Std Dev)	5.30 (4.735)	5.27 (4.130)	5.27 (4.130)	5.32 (4.117)
Median	4.0	4.0	4.0	4.1
P-value	0.0002	<0.0001	.0001	0.0001

Copied from Tables 14.2.2.1, 14.2.2.2, 14.2.2.3, and 14.2.2.4.
p-values are based on van Elteren tests adjusted for pooled center.

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Table 15 Overall Summaries of BM Frequency Rates [1] --- Protocol SC0131

Week Treatment Group Statistic	ITT Subjects with LOCF	ITT subjects without LOCF	ITT subjects who completed the study	Per Protocol Subjects
Baseline				
Placebo , n	119	119	115	116
Mean (Std Dev)	2.50 (1.703)	2.50 (1.703)	2.45 (1.697)	2.40 (1.429)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	120	120	106	111
Mean (Std Dev)	2.28 (1.131)	2.28 (1.131)	2.25 (1.133)	2.29 (1.145)
Median	2.0	2.0	2.0	2.0
P-value	0.5980	0.5980	0.8845	0.8188
Week 1				
Placebo , n	122	122	118	100
Mean (Std Dev)	3.71 (2.291)	3.71 (2.291)	3.75 (2.310)	3.65 (2.062)
Median	3.0	3.0	3.0	3.0
RU-0211 48 µg, n	116	116	105	101
Mean (Std Dev)	5.80 (4.326)	5.80 (4.326)	5.74 (4.335)	6.03 (4.378)
Median	5.0	5.0	5.0	5.0
P-value	0.0002	0.0002	0.0003	<0.0001
Overall p-value	<0.0001	<0.0001	<0.0001	<0.0001
Week 2				
Placebo , n	122	120	118	115
Mean (Std Dev)	3.71 (2.452)	3.74 (2.460)	3.74 (2.465)	3.72 (2.421)
Median	3.0	3.0	3.0	3.0
RU-0211 48 µg, n	116	114	105	106
Mean (Std Dev)	5.59 (3.745)	5.58 (3.736)	5.58 (3.626)	5.70 (3.732)
Median	5.0	5.0	5.0	5.0
P-value	<0.0001	<0.0001	<0.0001	<0.0001
Week 3				
Placebo , n	122	119	118	115
Mean (Std Dev)	3.50 (2.254)	3.54 (2.265)	3.54 (2.275)	3.51 (2.210)
Median	3.0	3.0	3.0	3.0
RU-0211 48 µg, n	116	108	104	102
Mean (Std Dev)	5.76 (4.624)	5.90 (4.668)	5.72 (4.040)	5.99 (4.766)
Median	5.0	5.0	5.0	5.0
P-value	0.0001	<0.0001	<0.0001	<0.0001
Week 4				
Placebo , n	122	118	117	111
Mean (Std Dev)	3.58 (2.260)	3.65 (2.255)	3.65 (2.265)	3.59 (2.168)
Median	3.0	3.0	3.0	3.0

RU-0211 48 µg, n	116	104	104	103
Mean (Std Dev)	6.02 (4.548)	6.01 (3.944)	6.01 (3.944)	6.01 (3.963)
Median	5.4	5.9	5.9	5.8
P-value	<0.0001	<0.0001	<0.0001	<0.0001

Copied from Tables 14.2.1.1, 14.2.1.2, 14.2.1.3, and 14.2.1.4.
p-values are based on van Elteren tests adjusted for pooled center.

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Table 16 Summary of Weekly Responder Status --- Protocol SC0131

Week Treatment Group Responder Status	ITT Subjects with LOCF	ITT subjects without LOCF	ITT subjects who completed the study	Per Protocol Subjects
Week 1				
Placebo , n	122	122	118	119
Full Responder	53/122 (43.4%)	53/122 (43.4%)	52/118 (44.1%)	48/100 (48.0%)
Moderate Responder	19/122 (15.6%)	19/122 (15.6%)	19/118 (16.1%)	19/100 (19.0%)
Non-Responder	50/122 (41.0%)	50/122 (41.0%)	47/118 (39.8%)	33/100 (33.0%)
RU-0211 48 µg, n	116	116	105	111
Full Responder	75/116 (64.7%)	75/116 (64.7%)	68/105 (64.8%)	71/101 (70.3%)
Moderate Responder	14/116 (12.1%)	14/116 (12.1%)	13/105 (12.4%)	13/101 (12.9%)
Non-Responder	27/116 (23.3%)	27/116 (23.3%)	24/105 (22.9%)	17/101 (16.8%)
P-value	0.0023	0.0023	0.0051	0.0025
Week 2				
Placebo , n	122	122	118	119
Full Responder	44/122 (36.1%)	44/120 (36.7%)	44/118 (37.3%)	43/115 (37.4%)
Moderate Responder	17/122 (13.9%)	17/120 (14.2%)	17/118 (14.4%)	16/115 (13.9%)
Non-Responder	61/122 (50.0%)	59/120 (49.2%)	57/118 (48.3%)	56/115 (48.7%)
RU-0211 48 µg, n	116	116	105	111
Full Responder	67/116 (57.8%)	66/114 (57.9%)	62/105 (59.0%)	62/106 (58.5%)
Moderate Responder	10/116 (8.6%)	10/114 (8.8%)	9/105 (8.6%)	10/106 (9.4%)
Non-Responder	39/116 (33.6%)	38/114 (33.3%)	34/105 (32.4%)	34/106 (32.1%)
P-value	0.0037	0.0054	0.0051	0.0069
Week 3				
Placebo , n	122	122	118	119
Full Responder	35/122 (28.7%)	35/119 (29.4%)	35/118 (29.7%)	34/115 (29.6%)
Moderate Responder	16/122 (13.1%)	16/119 (13.4%)	16/118 (13.6%)	16/115 (13.9%)
Non-Responder	71/122 (58.2%)	68/119 (57.1%)	67/118 (56.8%)	65/115 (56.5%)
RU-0211 48 µg, n	116	116	105	111
Full Responder	65/116 (56.0%)	62/108 (57.4%)	60/104 (57.7%)	59/102 (57.8%)
Moderate Responder	8/116 (6.9%)	8/108 (7.4%)	8/104 (7.7%)	7/102 (6.9%)
Non-Responder	43/116 (37.1%)	38/108 (35.2%)	36/104 (34.6%)	36/102 (35.3%)
P-value	0.0003	0.0003	0.0003	0.0003
Week 4				
Placebo , n	122	122	118	119
Full Responder	34/122 (27.9%)	34/118 (28.8%)	34/117 (29.1%)	33/111 (29.7%)
Moderate Responder	20/122 (16.4%)	20/118 (16.9%)	20/117 (17.1%)	20/111 (18.0%)
Non-Responder	68/122 (55.7%)	64/118 (54.2%)	63/117 (53.8%)	58/111 (52.3%)
RU-0211 48 µg, n	116	116	105	111
Full Responder	67/116 (57.8%)	62/104 (59.6%)	62/104 (59.6%)	62/103 (60.2%)
Moderate Responder	10/116 (8.6%)	10/104 (9.6%)	10/104 (9.6%)	10/103 (9.74%)
Non-Responder	39 /116 (33.6%)	32/104 (30.8%)	32/104 (30.8%)	31/103 (30.1%)
P-value	<0.0001	<0.0001	<0.0001	0.0002

Copied from Tables 14.2.4.1, 14.2.4.2, 14.2.4.3, and 14.2.4.4.
p-values are based on van Elteren tests adjusted for pooled center.

Table 17 Summary of Subjects with SBMs within 24 Hours after the First Study Drug Administration --- Protocol SC0131

SBMs within 24 Hours

(Intent-to-Treat Subjects without LOCF)

Treatment	SBM within 24 hours	p-value
Placebo	45/122 (36.9%)	0.0024
RU-0211 48 µg	68/120 (56.7%)	

Copied from Table 14.2.5.1

(Per Protocol Subjects)

Treatment	SBM within 24 hours	p-value
Placebo	45/119 (34.5%)	0.0106
RU-0211 48 µg	59/111 (53.2%)	

Copied from Table 14.2.5.2

Figure 18 Kaplan-Meier Curve for Time to First SBM (ITT subjects with LOCF) --- Protocol SC0131

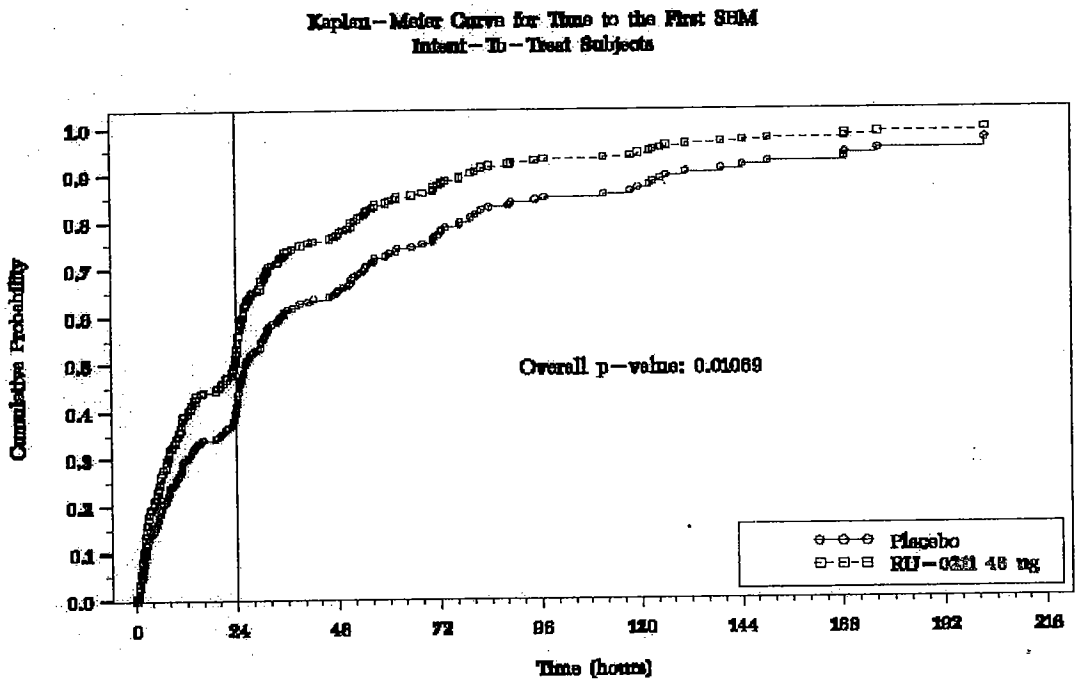

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Table 19 Overall Summary of Average Stool Consistency [1] --- Protocol SC0131

Week Treatment Group Statistic	ITT Subjects with LOCF	ITT subjects without LOCF	ITT subjects who completed the study	Per Protocol Subjects
Baseline				
Placebo , n	102	102	98	99
Mean (Std Dev)	2.74 (0.776)	2.74 (0.776)	2.74 (0.788)	2.74 (0.785)
Median	3.0	3.0	3.0	3.0
RU-0211 48 µg, n	102	102	88	93
Mean (Std Dev)	2.92 (0.783)	2.92 (0.783)	2.93 (0.797)	2.94 (0.790)
Median	3.0	3.0	3.0	3.0
P-value	0.0628	0.0628	0.0599	0.0344
Week 1				
Placebo , n	112	112	108	96
Mean (Std Dev)	2.61 (0.720)	2.61 (0.720)	2.60 (0.723)	2.58 (0.728)
Median	2.6	2.6	2.6	2.6
RU-0211 48 µg, n	113	113	100	98
Mean (Std Dev)	1.98 (0.975)	1.98 (0.975)	1.98 (0.982)	1.97 (0.941)
Median	2.0	2.0	2.0	2.0
P-value	<0.0001	<0.0001	<0.0001	<0.0001
Week 2				
Placebo , n	117	108	107	103
Mean (Std Dev)	2.58 (0.781)	2.62 (0.758)	2.63 (0.761)	2.62 (0.768)
Median	2.6	2.6	2.7	2.6
RU-0211 48 µg, n	114	93	90	90
Mean (Std Dev)	1.80 (0.919)	1.76 (0.879)	1.77 (0.872)	1.78 (0.885)
Median	1.8	1.8	1.7	1.8
P-value	<0.0001	<0.0001	<0.0001	<0.0001
Week 3				
Placebo , n	119	105	104	101
Mean (Std Dev)	2.60 (0.838)	2.58 (0.861)	2.58 (0.864)	2.58 (0.871)
Median	2.7	2.7	2.7	2.7
RU-0211 48 µg, n	114	91	89	88
Mean (Std Dev)	1.84 (0.906)	1.84 (0.858)	1.87 (0.840)	1.83 (0.870)
Median	1.9	1.8	1.8	1.8
P-value	<0.0001	<0.0001	<0.0001	<0.0001
Week 4				
Placebo , n	121	99	98	94
Mean (Std Dev)	2.50 (0.690)	2.48 (0.688)	2.47 (0.689)	2.47 (0.682)
Median	2.5	2.5	2.5	2.5
RU-0211 48 µg, n	115	89	89	89
Mean (Std Dev)	1.81 (0.894)	1.85 (0.837)	1.85 (0.837)	1.85 (0.837)
Median	1.9	1.9	1.9	1.9
P-value	<0.0001	<0.0001	<0.0001	<0.0001

Copied from Tables 14.2.6.1, 14.2.6.2, 14.2.6.3, and 14.2.6.4.
p-values are based on van Elteren tests adjusted for pooled center.

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Table 20 Overall Summary of Average Degree of Straining [1] --- Protocol SC0131

Week Treatment Group Statistic	ITT Subjects with LOCF	ITT subjects without LOCF	ITT subjects who completed the study	Per Protocol Subjects
Baseline				
Placebo , n	101	101	97	98
Mean (Std Dev)	2.40 (0.880)	2.40 (0.880)	2.39 (0.890)	2.40 (0.885)
Median	2.5	2.5	2.5	2.5
RU-0211 48 µg, n	102	102	88	93
Mean (Std Dev)	2.48 (0.848)	2.48 (0.848)	2.51 (0.864)	2.52 (0.844)
Median	2.5	2.5	2.6	2.6
P-value	0.4905	0.4905	0.4637	0.3967
Week 1				
Placebo , n	112	112	108	96
Mean (Std Dev)	2.11 (0.914)	2.11 (0.914)	2.07 (0.904)	2.05 (0.907)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	113	113	100	98
Mean (Std Dev)	1.64 (0.847)	1.64 (0.847)	1.63 (0.845)	1.58 (0.817)
Median	1.7	1.7	1.6	1.6
P-value	<0.0001	<0.0001	0.0002	0.0001
Week 2				
Placebo , n	117	107	106	102
Mean (Std Dev)	2.14 (0.940)	2.14 (0.936)	2.15 (0.939)	2.14 (0.950)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	114	93	90	90
Mean (Std Dev)	1.47 (0.883)	1.37 (0.853)	1.40 (0.852)	1.40 (0.861)
Median	1.4	1.3	1.3	1.3
P-value	<0.0001	<0.0001	<0.0001	<0.0001
Week 3				
Placebo , n	119	104	103	100
Mean (Std Dev)	2.14 (0.962)	2.10 (0.978)	2.09 (0.979)	2.10 (0.997)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	114	91	89	88
Mean (Std Dev)	1.49 (0.914)	1.44 (0.909)	1.45 (0.904)	1.43 (0.912)
Median	1.5	1.3	1.3	1.3
P-value	<0.0001	<0.0001	<0.0001	<0.0001
Week 4				
Placebo , n	121	98	97	93
Mean (Std Dev)	2.09 (0.874)	2.05 (0.845)	2.05 (0.850)	2.06 (0.864)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	114	88	88	88
Mean (Std Dev)	1.50 (0.870)	1.49 (0.866)	1.49 (0.866)	1.49 (0.866)
Median	1.5	1.5	1.5	1.5
P-value	<0.0001	<0.0001	<0.0001	<0.0001

Copied from Tables 14.2.7.1, 14.2.7.2, 14.2.7.3, and 14.2.7.4.
p-values are based on van Elteren tests adjusted for pooled center.

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Table 21 Overall Summary of Average Severity of Constipation [1] --- Protocol SC0131

Week Treatment Group Statistic	ITT Subjects with LOCF	ITT subjects without LOCF	ITT subjects who completed the study	Per Protocol Subjects
Baseline				
Placebo , n	122	122	118	119
Mean (Std Dev)	3.07 (0.794)	3.07 (0.794)	3.06 (0.798)	3.08 (0.794)
Median	3.0	3.0	3.0	3.0
RU-0211 48 µg, n	119	119	105	110
Mean (Std Dev)	3.13 (0.671)	3.13 (0.671)	3.13 (0.694)	3.14 (0.684)
Median	3.0	3.0	3.0	3.0
P-value	0.8528	0.8528	0.6895	0.8168
Week 1				
Placebo , n	119	119	116	97
Mean (Std Dev)	2.49 (1.007)	2.49 (1.007)	2.48 (1.000)	2.39 (0.919)
Median	3.0	3.0	3.0	2.0
RU-0211 48 µg, n	114	114	103	98
Mean (Std Dev)	1.97 (1.163)	1.97 (1.163)	2.00 (1.172)	1.86 (1.149)
Median	2.0	2.0	2.0	2.0
P-value	0.0003	0.0003	0.0005	0.0004
Week 2				
Placebo , n	122	108	106	105
Mean (Std Dev)	2.41 (1.010)	2.43 (1.025)	2.42 (1.022)	2.43 (1.027)
Median	2.0	2.5	2.5	2.0
RU-0211 48 µg, n	116	109	102	102
Mean (Std Dev)	1.78 (1.120)	1.76 (1.138)	1.75 (1.123)	1.76 (1.170)
Median	2.0	2.0	2.0	2.0
P-value	<0.0001	<0.0001	<0.0001	0.0002
Week 3				
Placebo , n	122	109	108	105
Mean (Std Dev)	2.48 (1.108)	2.52 (1.059)	2.52 (1.063)	2.50 (1.066)
Median	3.0	3.0	3.0	3.0
RU-0211 48 µg, n	117	103	99	96
Mean (Std Dev)	1.91 (1.103)	1.90 (1.151)	1.89 (1.133)	1.86 (1.166)
Median	2.0	2.0	2.0	2.0
P-value	<0.0001	<0.0001	<0.0001	0.0001
Week 4				
Placebo , n	122	109	108	103
Mean (Std Dev)	2.52 (1.130)	2.52 (1.085)	2.52 (1.089)	2.49 (1.074)
Median	3.0	3.0	3.0	3.0
RU-0211 48 µg, n	117	102	100	100
Mean (Std Dev)	1.94 (1.198)	1.93 (1.237)	1.91 (1.232)	1.92 (1.245)
Median	2.0	2.0	2.0	1.5
P-value	<0.0001	0.0003	<0.0001	0.0007

Follow-up				
Placebo , n	118	118	116	116
Mean (Std Dev)	2.57 (0.920)	2.57 (0.920)	2.55 (0.917)	2.57 (0.925)
Median	3.0	3.0	3.0	3.0
RU-0211 48 µg, n	113	113	105	106
Mean (Std Dev)	2.55 (1.086)	2.55 (1.086)	2.55 (1.083)	2.58 (1.086)
Median	3.0	3.0	3.0	3.0
P-value	0.8532	0.8532	0.6860	0.7227

Copied from Tables 14.2.9.1, 14.2.9.2, 14.2.9.3, and 14.2.9.4.
p-values are based on van Elteren tests adjusted for pooled center.

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Table 22 Overall Summary of Global Assessment of Treatment Effectiveness [1] --- Protocol SC0131

Week Treatment Group Statistic	ITT Subjects with LOCF	ITT subjects without LOCF	ITT subjects who completed the study	Per Protocol Subjects
Week 1				
Placebo , n	119	119	116	97
Mean (Std Dev)	0.97 (1.008)	0.97 (1.008)	0.98 (1.013)	1.05 (0.961)
Median	1.0	1.0	1.0	1.0
RU-0211 48 µg, n	114	114	103	98
Mean (Std Dev)	1.76 (1.292)	1.76 (1.292)	1.73 (1.292)	1.84 (1.274)
Median	2.0	2.0	2.0	2.0
P-value	<0.0001	<0.0001	<0.0001	<0.0001
Week 2				
Placebo , n	122	108	106	105
Mean (Std Dev)	0.96 (1.209)	0.94 (1.233)	0.94 (1.241)	0.95 (1.243)
Median	0.0	0.0	0.0	0.0
RU-0211 48 µg, n	116	108	102	101
Mean (Std Dev)	1.78 (1.286)	1.77 (1.309)	1.76 (1.291)	1.75 (1.330)
Median	2.0	2.0	2.0	2.0
P-value	<0.0001	<0.0001	<0.0001	<0.0001
Week 3				
Placebo , n	122	110	109	106
Mean (Std Dev)	0.90 (1.202)	0.89 (1.168)	0.90 (1.1760)	0.92 (1.177)
Median	0.0	0.0	0.0	0.0
RU-0211 48 µg, n	117	101	98	96
Mean (Std Dev)	1.68 (1.298)	1.69 (1.325)	1.70 (1.302)	1.74 (1.332)
Median	2.0	2.0	2.0	2.0
P-value	<0.0001	<0.0001	<0.0001	<0.0001
Week 4				
Placebo , n	122	109	108	103
Mean (Std Dev)	0.94 (1.228)	0.94 (1.201)	0.94 (1.206)	0.95 (1.208)
Median	0.0	0.0	0.0	0.0
RU-0211 48 µg, n	117	102	100	100
Mean (Std Dev)	1.81 (1.364)	1.80 (1.393)	1.80 (1.378)	1.82 (1.395)
Median	2.0	2.0	2.0	2.0
P-value	<0.0001	<0.0001	<0.0001	<0.0001
Follow-up				
Placebo , n	118	118	116	116
Mean (Std Dev)	0.94 (1.242)	0.94 (1.242)	0.95 (1.250)	0.95 (1.250)
Median	0.0	0.0	0.0	0.0

RU-0211 48 µg, n	114	114	104	106
Mean (Std Dev)	1.89 (1.444)	1.89 (1.444)	1.93 (1.423)	1.94 (1.440)
Median	2.0	2.0	2.0	2.0
P-value	<0.0001	<0.0001	<0.0001	<0.0001

Copied from Tables 14.2.11.1, 14.2.11.2, 14.2.11.3, and 14.2.11.4.
p-values are based on van Elteren tests adjusted for pooled center.

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Table 23 Overall Summary of Abdominal Bloating [1] --- Protocol SC0131

Week Treatment Group Statistic	ITT Subjects with LOCF	ITT subjects without LOCF	ITT subjects who completed the study	Per Protocol Subjects
Baseline				
Placebo , n	122	122	118	119
Mean (Std Dev)	2.12 (0.967)	2.12 (0.967)	2.12 (0.971)	2.13 (0.965)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	120	120	105	110
Mean (Std Dev)	2.17 (0.929)	2.17 (0.929)	2.15 (0.934)	2.16 (0.920)
Median	2.0	2.0	2.0	2.0
P-value	0.8660	0.8660	0.9872	0.9967
Week 1				
Placebo , n	119	119	116	97
Mean (Std Dev)	1.73 (0.980)	1.73 (0.980)	1.72 (0.965)	1.75 (0.936)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	114	114	103	98
Mean (Std Dev)	1.57 (1.030)	1.57 (1.030)	1.55 (1.036)	1.54 (0.997)
Median	1.0	1.0	1.0	1.0
P-value	0.2928	0.2928	0.2150	0.1384
Week 2				
Placebo , n	122	108	106	105
Mean (Std Dev)	1.79 (1.093)	1.75 (1.086)	1.74 (1.089)	1.77 (1.094)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	116	109	102	102
Mean (Std Dev)	1.44 (1.049)	1.45 (1.067)	1.42 (1.048)	1.40 (1.055)
Median	1.0	1.0	1.0	1.0
P-value	0.0207	0.0532	0.0392	0.0126
Week 3				
Placebo , n	122	110	109	106
Mean (Std Dev)	1.91 (1.083)	1.94 (1.086)	1.93 (1.086)	1.92 (1.093)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	117	103	99	96
Mean (Std Dev)	1.57 (1.069)	1.51 (1.083)	1.49 (1.063)	1.44 (1.064)
Median	2.0	2.0	2.0	1.0
P-value	0.0310	0.0045	0.0042	0.0016
Week 4				
Placebo , n	122	109	108	103
Mean (Std Dev)	1.75 (1.080)	1.76 (1.053)	1.76 (1.058)	1.77 (1.040)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	117	102	100	100
Mean (Std Dev)	1.49 (1.096)	1.47 (1.132)	1.47 (1.123)	1.46 (1.141)
Median	1.0	1.0	1.0	1.0
P-value	0.0987	0.0580	0.0731	0.0379

Follow-up				
Placebo , n	118	118	116	116
Mean (Std Dev)	1.77 (1.073)	1.77 (1.073)	1.77 (1.074)	1.78 (1.078)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	113	113	105	106
Mean (Std Dev)	1.85 (1.159)	1.85 (1.159)	1.85 (1.175)	1.85 (1.178)
Median	2.0	2.0	2.0	2.0
P-value	0.3647	0.3647	0.3736	0.4170

Copied from Tables 14.2.12.1, 14.2.12.2, 14.2.12.3, and 14.2.12.4.
p-values are based on van Elteren tests adjusted for pooled center.

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Table 24 Overall Summary of Abdominal Discomfort [1] --- Protocol SC0131

Week Treatment Group Statistic	ITT Subjects with LOCF	ITT subjects without LOCF	ITT subjects who completed the study	Per Protocol Subjects
Baseline				
Placebo , n	122	122	118	119
Mean (Std Dev)	1.97 (0.962)	1.97 (0.962)	1.97 (0.951)	1.98 (0.948)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	120	120	106	111
Mean (Std Dev)	1.93 (0.936)	1.93 (0.936)	1.94 (0.954)	1.93 (0.941)
Median	2.0	2.0	2.0	2.0
P-value	0.6635	0.6635	0.8693	0.6565
Week 1				
Placebo , n	119	119	116	97
Mean (Std Dev)	1.34 (0.994)	1.34 (0.994)	1.31 (0.982)	1.36 (0.981)
Median	1.0	1.0	1.0	1.0
RU-0211 48 µg, n	114	114	103	98
Mean (Std Dev)	1.35 (1.072)	1.35 (1.072)	1.32 (1.068)	1.29 (1.015)
Median	1.0	1.0	1.0	1.0
P-value	0.8005	0.8005	0.9020	0.5004
Week 2				
Placebo , n	122	108	106	105
Mean (Std Dev)	1.41 (1.035)	1.44 (1.026)	1.42 (1.032)	1.45 (1.028)
Median	1.0	2.0	1.5	2.0
RU-0211 48 µg, n	116	109	102	102
Mean (Std Dev)	1.09 (1.047)	1.09 (1.059)	1.07 (1.017)	1.05 (1.057)
Median	1.0	1.0	1.0	1.0
P-value	0.0245	0.0140	0.0154	0.0046
Week 3				
Placebo , n	122	110	109	106
Mean (Std Dev)	1.63 (1.122)	1.65 (1.137)	1.64 (1.135)	1.62 (1.142)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	117	103	99	96
Mean (Std Dev)	1.27 (1.056)	1.22 (1.066)	1.20 (1.040)	1.17 (1.043)
Median	1.0	1.0	1.0	1.0
P-value	0.0169	0.0045	0.0048	0.0033
Week 4				
Placebo , n	122	109	108	103
Mean (Std Dev)	1.52 (1.038)	1.52 (1.015)	1.52 (1.018)	1.52 (1.008)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	117	102	100	100
Mean (Std Dev)	1.23 (1.062)	1.22 (1.087)	1.20 (1.054)	1.21 (1.094)
Median	1.0	1.0	1.0	1.0
P-value	0.0445	0.0167	0.0193	0.0172

Follow-up				
Placebo , n	118	118	116	116
Mean (Std Dev)	1.59 (1.023)	1.59 (1.023)	1.59 (1.030)	1.60 (1.029)
Median	2.0	2.0	2.0	2.0
RU-0211 48 µg, n	113	113	105	106
Mean (Std Dev)	1.67 (1.106)	1.67 (1.106)	1.65 (1.109)	1.67 (1.119)
Median	2.0	2.0	2.0	2.0
P-value	0.3701	0.3701	0.5937	0.5378

Copied from Tables 14.2.13.1, 14.2.13.2, 14.2.13.3, and 14.2.13.4.
p-values are based on van Elteren tests adjusted for pooled center.

Table 25 Summary of Demographic and Baseline Characteristics --- Protocol SC9921

Characteristics	Placebo (N=33)	(ITT Subjects) RU-0211 24 µg (N=29)	48 µg (N=32)	72 µg (N=33)	Among Treatment p-value
Sex					0.399
Male	2 (6.1%)	1 (3.4%)	4 (10.8%)	5 (15.2%)	
Female	31 (93.9%)	28 (96.6%)	28 (89.2%)	28 (84.8%)	
Race					0.598
White	28 (84.8%)	25 (86.2%)	29 (90.6%)	27 (81.8%)	
Black	3 (9.1%)	4 (13.8%)	2 (6.3%)	6 (18.2%)	
Hispanic	1 (3.0%)		0 (0.0%)		
Other Races	1 (3.0%)		1 (3.1%)		
Age (months)					0.478
Mean (SD)	46.8 (12.4)	47.6 (11.5)	49.3 (12.1)	49.4 (13.8)	
Height (in)					0.494
Mean (SD)	64.8 (3.24)	64.8 (2.10)	65.8 (3.09)	65.6 (2.76)	
Flexible Sigmoidoscopy					0.681
No	24 (72.7%)	18 (62.1%)	21 (65.6%)	24 (72.7%)	
Yes	9 (27.3%)	11 (37.9%)	11 (34.4%)	9 (27.3%)	

Copied from Table 11.2.1.
P-values are based on ANOVA for continuous variables and CMH for categorical variables with investigative site used as a factor.

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/s/

Milton Fan
12/15/2005 04:43:34 PM
BIOMETRICS

Stella Grosser
12/16/2005 03:47:40 PM
BIOMETRICS
concur

S. Edward Nevius
12/16/2005 05:14:32 PM
BIOMETRICS
Concur with review.

Statistical Review - Carcinogenicity Studies

NDA: 21-908

Applicant: Sucampo Pharmaceuticals, Inc.

Name of Drug: Etrevia

Documents Reviewed: Electronic

Pharmacology Reviewer: S. Chakder, Ph.D.

Date Submitted: March 31, 2005

Statistical Reviewer: Mushfiqur Rashid, Ph.D.

I. **Background**: In this NDA submission, two animal carcinogenicity studies (one in B6C3F1 mice and one in Sprague-Dawley rats) were included. These two studies were intended to assess the carcinogenic potential of Etrevia (RU-0211) in the diet of B6C3F1 mice and Sprague-Dawley rats when administered orally using some selected dose levels. Dr. S. Chakder, HFD-180, who is the reviewing pharmacologist, requested the Division of Biometrics II to perform the statistical review and evaluation of this submission.

This review is organized as follows: Section 2 describes the statistical methodology utilized in this submission; Section 3 contains the analysis of the mouse study; Section 4 contains the analysis of the rat study. Section 5 summarizes the conclusions of this submission.

2. Statistical Methodology:

This reviewer performed an independent analysis of the carcinogenicity data submitted by the sponsor. This reveiwer's analysis conformed to the Food and drug Administration's Guidance for Industry: Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals (May 2001). Because there are two control groups in this submission, this reviewer combined the two control groups as a single group at the advice of the reviewing pharmacologist. The analysis was primarily conducted using eReview of Animal Carcinogenicity, a review tool developed and utilized by CDER reviewers.

Mortality Analysis: Tests for homogeneity and dose mortality trends were conducted using the survival analysis methods described by Cox (1972), and Gehan (1965). Note that the Gehans's test weights early failures more heavily.

Trend Tests: This reviewer conducted the trend tests on tumor incidence rates using the method described by Peto et al. (1980). and the method of exact permutation trend test, developed by the Division of Biometrics II. The sponsor classified tumors as fatal or incidental, and the tumors were analyzed via the death-rate and prevalence methods, respectively. A combined test was utilized to analyze tumors classified as both fatal and incidental. The method of exact permutation trend test was used to counter underestimation of p-values when tumor occurrence across the treatment groups was small. All test are performed separately for males and females for both species.

Multiple Testing Adjustment: A rule proposed by Haseman could be used to adjust [illegible] multiple testings. A similar rule proposed by the Division of Biometrics, CDER/FDA was used in this review. This rule states that in order to keep the overall false-positive rate at the nominal level of approximately ten percent, tumor types with a spontaneous tumor rate of no more than one percent should be tested at 0.025 level, otherwise the level should be set at 0.005.

Evaluation of Validity of the Design of the Study

An evaluation of validity of the study design was conducted in a negative study (that is, an analysis did not indicate any tumor type with a significant positive linear trend) before drawing the conclusion that the drug was not carcinogenic in rodents. It is important to look into the following two issues in the evaluation as pointed out in the paper by Haseman (Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies, Environmental Health Perspectives, Vol. 58, pp 385-392, 1984). The two issues are:

(i) Were enough animals exposed, for a sustained amount of time, to the risk of late developing tumor ?

(ii) Were dose levels high enough to pose a reasonable tumor challenge to the animals ?

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

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

Haseman (Issues in carcinogenicity testing: Dose selection, Fundamental and Applied Toxicology, Vol. 5, pp 66-78, 1985) did an investigation on the first issue. He gathered data from 21 studies using Fischer 344 rats and B6C3F1 mice conducted at the National Toxicology Program (NTP). It was found that, on an average, approximately 50% of the animals in the high dose group survived the two-year study period. Also, in a personal communication with Dr. Karl Lin of Division of Biometrics II/OEB/CDER, Haseman suggested that, as a rule of thumb, a 50%

survival of 50 initial animals in the high dose group, between weeks 80-90, would be considered as a sufficient number of animals under an adequate exposure.

In addition, Chu, Cueto, and Ward (Factors in the evaluation of 200 national cancer institute carcinogen bioassay, Journal of Toxicology and environmental Health. Vol. 8, pp 251-280, 1981), suggested that " To be considered adequate, an experiment that has not shown a chemical to be carcinogenic should have groups of animals with greater than 50% survival at one-year."

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

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

i) " A dose is considered adequate if there is a detectable loss in weight gain of up to 10 % in a dosed group relative to the controls."

ii) " The administered dose is also considered an MTD if dosed animals exhibit clinical signs or severe histopathologic toxic effects attributed to the chemical."

iii) " In addition, doses are considered adequate if the dosed animals show a slightly increased mortality compared to the controls."

Note that only one of the above three criteria is needed.

3. The Mouse Study

3.1 Design

Two separate experiments, one in male and one in female mice, were conducted over a period of 104 weeks. In each of the studies there were four treated groups 25, 75, 200, and 500 µg/kg/day and one control group. For each sex, 275 hundred B6C3F1 mice (approximately days of age) were randomly divided into equal groups of 55 animals each to form the treatment groups and the control groups. The dose levels for the treated groups were 25, 75, 200 and 500 µg/kg/day for the low, medium, medium high and high dose groups, respectively. The dose level of control groups was 0 mg/kg/day. The purpose of this study was to fully evaluate any deviations from the normal or spontaneous lifetime incidence of neoplasms in mice due to drug effect.

All mice were examined daily prior to and following dosing for mortality and once per week for physical signs. Beginning in Drug Week 26, all animals were palpated for masses every 4 weeks to provide information regarding the onset of possible neoplasms for use in statistical analyses. Body weights were recorded pretest, once in Drug Week 1, generally twice per week through Drug week 13, and then once per week thereafter. Opthalmic examinations were performed pretest on all animals and in drug Weeks 52 and 93 on Control and high dose animals.

Mice sacrificed prior to or at the termination of the study were anesthetized and euthanized by exsanguination prior to necropsy. Complete necropsies, including examination and collection of tissues from an extensive list, were done except one exception. One female in Control group 1 escaped during a cage change in drug week 49 and was never found. At the discretion of the pathologist, blood samples were collected from animals euthanized prior to the terminal necropsy to aid in the determination of possible leukemias.

3.2 Sponsor's Analysis

Survival Data Analysis:

The sponsor presented mortality data for the main study animals. It was concluded that there was no significant trend or increase or decrease in mortality in treated groups of either sex in this study.

Tumor Data Analysis:

In the males, there was no significant trend or either significant increase or decrease in any neoplastic lesion.

In the females, there was no significant trend on any neoplastic lesion. There was an isolated in group 3 malignant lymphoma incidence rate compared to the control group. All other treated groups had similar incidence rates as in the control group in this case. As a result, this increase is an intermediate group is not considered to be treatment related.

Sponsor's Conclusions: The sponsor concluded that the daily oral administration of RU-0021at 25, 75, 200, and 500 µg/kg/day to male and female mice for approximately 105 weeks was well tolerated. With increasing doses of RU-0211, there were no treatment related or statistically significant (p-value >0.05) effects on mortality. There was no statistically significant (p-value according to the Division's p-value adjustment rule) evidence of trend in the incidence of any tumor type in either sex of mice. There were no treatment related neoplastic changes.

3.3 Reviewer's Analysis

At the termination of drug administration, mortality in males was 27%, 35%, 20%, 40% and 36% for the control, 25, 75, 200, and 500 µg/kg/day. Similarly, the percent mortality for females at the termination of drug administration was 29%, 24%, 35%, 40%, and 24% for the respective ascending dose groups. This reviewer conducted an investigation of the mortality among dose groups via Kaplan-Meier product limit survival curves depicted in Figure 1a and Figure1b. For

both male and female mouse, the test did not yield any significant dose mortality trends. Results of tests of homogeneity and trend are displayed in Table A.1 and Table A.2 in the Appendix (A)

Table A.3 and table A.4 depict results of the linear trend tests for each tumor type by gender. There were no incidence rates of tumor types with p-values less than .05 in the nouse studies.

Evaluation of Validity of the Design of the Mouse Study:

The reviewer's analysis results show that in both male and female mouse studies, there is no statistically significant positive dose-response relationship in any tumor type tested. However, before drawing the conclusion that the drug is not carcinogenic in mouse, it is important to evaluate the validity of the negative study for the mouse study

We will now investigate the validity of the CD-1 mice carcinogenicity study, in the light of the guidelines mentioned in Section 2.

The following are summary survival data of mice in the high dose group.

Table 3.3: Survival Rates for the High Dose Group

Sex	End of 52 weeks	End of 78 weeks	End of 93 weeks	End of 103 weeks
Male	100%	95%	91%	76%
Female	100%	95%	86%	64%

From the above summary data, it can be concluded that more than 50% of the animal were alive in the high dose group at the beginning of Week 90 suggesting that a sufficient number of animals with adequate exposure. From the above summary survival data, and the survival criteria mentioned in section 2, it can be concluded that there were enough mice exposed for sufficient amount of time to the drug.

To evaluate adequacy of dose, a summary of the body weight data for male and female mouse was generated and displayed in the following tables:

Table 3.4: Mean Body Weight(gms) for Male

Group	Day 0 of study	End of Study	Weight Gain	% of Control
Control	25.3	32.9	7.6	
low	25.3	32.2	6.9	91%
Medium	25.4	32.6	7.2	95%
Medium High	25.6	31.9	6.3	83%
High	25.7	33.9	8.2	108%

Table 3.5: Mean body weight(gms) for Female

Group	Day 0 of study	End of Study	Weight Gain	% of Control
Control	19.2	32.9	13.7	
Low	18.7	32.2	13.5	99%
Medium	19.2	32.6	13.4	98%
Medium High	19.5	31.9	12.4	91%
High	19.4	33.9	14.5	106%

From the above tables, it can be concluded that relative to the control, male mouse had an increment of weight gain in the high dose group equal to 8% whereas female rats had a decrement of weight in the high dose group equal to 6%, respectively. The increased weight gain in both male and female mouse suggests that the dosage for the high dose group may be inadequate.

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

Table 3.6: Mortality Rates at the End of the Experiment

Sex \Dose	Control	High Dose
Male	29%	24%
Female	27%	36%

From the above table we see that both for male and female mice, the mortality rate of the high dose group is lower than that of the combined control groups. The decreased mortality rates at the high dose group relative to the combined control suggests that an inadequacy of doses for both male and female mice.

4. The Rat Study

4.1 Design:

A study was conducted to determine the carcinogenic potential of Etrevia (RU-0211) when administered orally to rats for approximately 104 weeks at doses of 20, 100, or 400 μg/kg bid of the formulation.

Two hundred sixty female and 260 male rats at study initiation were randomly assigned to one control (0 mg/kg/bid) groups and three treated groups. Sixty five Sprague-Dawley rats of either sex were assigned in each treated group and one control group. The treated groups were given RU-0211 20, 100, or 400 μg/kg/day. The purpose of this study was to fully evaluate any deviations from the normal or spontaneous lifetime incidence of neoplasms in rats due to drug effect.

The rats observed daily for mortality and at least once weekly for physical signs. Beginning Week 26, all animals were palpated for masses every four weeks to provide information regarding the onset of possible neoplasms for use in statistical analyses. Body weights were recorded pretest, once in drug Week 1, twice per week in drug weeks 2 through 13, and once per week in Drug weeks 14 through 104. Opthalmic examinations were performed on all animals pretest and on animals in one of the control groups and the high dose group in drug weeks 51 and 101.

Rats sacrificed prior to or at study termination were anesthetized and euthanized by exsanguination prior to necropsy. Complete necropsies, including examination and collection of tissues from an extensive list, were done on all animals. At the discretion of the pathologist, blood samples were collected from animals euthanized prior to the terminals necropsy to aid in the determination.

Following routine fixation and processing, sections of numerous tissues from all animals were stained with hematoxylin and eosin and examined micoroscopically. At the discretion of the pathologist, tissues with grossly noted changes were also similarly processed, stained, and examined microscopically. Special stains performed as necessary on selected tissues to aid in the microscopic interpretation.

4.2 Sponsor's Analysis

Survival Data Analysis:

The sponsor concluded that there was no significant positive trend or increase in adjusted mortality in the males. In fact, 20 mug/kg/day group in the males showed a significantly lower adjusted mortality than the control. There was a significant negative trend in adjusted mortality rates in the females with 400 µg/kg/day showing a significantly lower rate in adjusted mortality compared to the control group.

Tumor Data Analysis:

There were no consistent treatment related increase or trend in any of the neoplastic lesions in either of the two sexes. If one assumes that the hyperplasia of the nonglandular stomach is a precursor to the squamous cell papilloma and carcinoma of the same organs, then there are

significant positive trends with significant increases in all treated groups in both sexes in these combined incidences.

There were significant positive trends in nonglandular stomach hyperplasia of both sexes. In both sexes, this was associated with significant increases in all treated groups versus the control group. The basophilic cellular alteration of the liver showed a significant increase at the 400/mu/kg/day group in the females only.

However, there were significant (according to the Division's p-value adjustment rule) positive trends in liver/adenoma (hepatocellular) in female rats and testis/B interstitial in male rats.

Sponsor's Conclusions:

There was no detrimental effect on adjusted survival in either sex of this study. In fact, the 400 mg/kg/day female group showed a significantly increased adjusted survival compared to control group causing a significant negative trend in survival in this sex.

The sponsor reported that there were no consistent treatment related increase or trend in any of the neoplastic lesions in either of the two sexes. However, there were significant (according to the Division's p-value adjustment rule) positive trends in liver/adenoma (hepatocellular) in female rats and testis/B interstitial in male rats.

4.3 Reviewer's analysis

Mortality Data Analysis:

At the termination of drug administration, mortality in males was 45%, 32%, 46%, and 49% for the control, 20 mg, 100 mg, 400 mg /kg/bid groups. Similarly, the percent mortality for females at the termination of drug administration was 68%, 63%, 60%, and 46% for the respective ascending dose groups. Figure 2a and 2b present the plots of Kaplan-Meier estimates of the survival distributions of the treatment groups for male and female rats, respectively. The tests show that the survival curves are homogeneous. However, there is a significant dose-mortality trend in the female rats. Results of these tests are displayed in Table A5 and table A.6.

Tumor Incidence Rates Analysis:

Table A.7 and Table A.8 depict results of the linear trend tests for each tumor type by gender. The incidence rates of the tumor types with p-values less than 0.05 are listed in Table 4.1 and Table 4.2.

Table 4.1 (Reviewer) Tumor Incidence rates (female) with P-value less than 0.05

Organ	Tumor	Overall	Tumor	Control	Low	Medium	High	P-value

Name	name	Tumor type	Rate in Control Group (%)					
Hemato Neoplasia	M-Sarcoma, histocytic	rare	0%	0	1	1	4	Exact 0.0277>0.025
liver	Adenoma hepatocellular	rare	0%	0	1	1	5	Exact .0031<0.025

From the above table, on the basis of the Division's p-value adjustment rule we only see significant positive trend in the incidence of the tumor type liver/adenoma in female rats

Table 4.2 (Reviewer) Tumor Incidence rates (male) with P-value less than 0.05

Organ Name	Tumor name	Overall Tumor type	Tumor Rate in Control Group	Control 1	Low	Medium	High	P-value
Cavity, Thoracic	Osteosarcoma	Common	1.54%	1	0	0	0	Exact 0.042(>0.005)
Testis	B - Interstitital	Common	3%	2	4	[illegible]	10	0.0006 (<0.005)

From the above table, on the basis of the Division's p-value adjustment rule we do not see any significant positive trend in the incidence of the tumor type thyroid/cavity osteosarcoma in male rats. However, on the basis of the Division's p-value adjustment rule there is a significant positive trend in the incidence of the tumor type Testis/ B –Interstitital in male rats.

5. Summary

a) The Mouse Study

For the mortality data analysis, the tests show that the survival curves are not statistically significant at 0.05 level. For tumor incidence rate analysis, on the basis of Division's p-value adjustment rule, no significant positive trend in the incidence of any tumor type is detected.

Using the criteria for evaluating the validity of experimental designs of negative studies proposed by experts in the field, it may be concluded that there is an inadequacy of doses.

b) The Rat Study

For the mortality data analysis, the tests show that for both male and female rats, the survival curves are not statistically significant at 0.05 level. However, there is a significant dose mortality trend in the female rats.

On the basis of the Division's p-value adjustment rule there is a significant positive trend in the incidence of the tumor type Testis/ B –Interstitital in male rats and liver/adenoma-hepatocellular in female rats.

M. Mushfiqur Rashid, Ph.D.
Mathematical Statistician

Concur: Dr. Lin

Appendix

Figure1a: Kaplan-Meier Survival Functions for Male Mice

(NDA 21908)

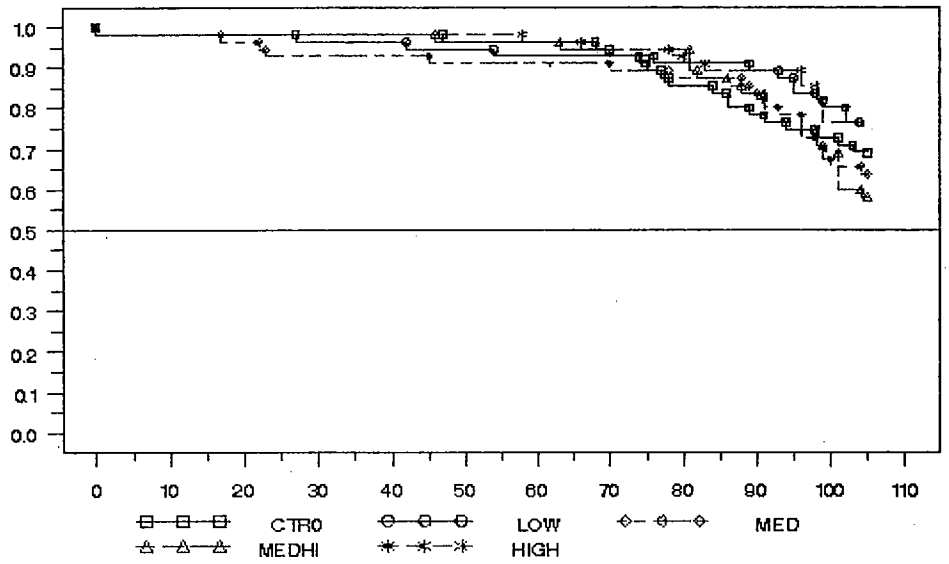

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Figure1b: Kaplan-Meier Survival Functions for Female Mice

(NDA 21908)

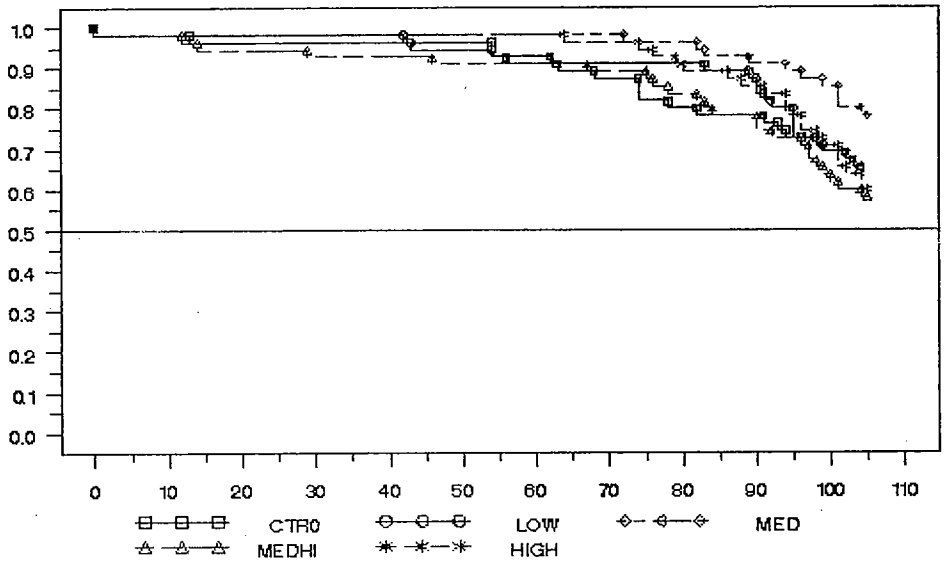

Table A.1: Analysis of Dose-Mortality Trend

Species: Mouse, Sex: Male, NDA 21908

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test	5.0400	0.1689	4.6556	0.1988

Depart from Trend				
Dose-Mortality Trend	0.4210	0.5164	0.5701	0.4502
Homogeneity	5.4610	0.2432	5.2256	0.2649

Table A.2: Analysis of Dose-Mortality Trend

Species: Mouse, Sex: Female, NDA 21908

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	5.3077	0.1506	5.9427	0.1144
Dose-Mortality Trend	1.1133	0.2914	0.7238	0.3949
Homogeneity	6.4210	0.1698	6.6665	0.1546

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Table A.3: Report on Trend Test – Male Mice

Organ Name	Tumor Name	DOSE1	DOSE2	DOSE3	DOSE4	DOSE5	P-Value
ADRENAL, CORTEX	B-ADENOMA	3	1	2	2	2	0.5368
ADRENAL, CORTEX	B-ADENOMA, SUBCAPSULAR CELL	0	1	1	0	2	0.144
DUODENUM	M-CARCINOMA	0	0	0	1	0	0.4179
EPIDIDYMIS	M-SARCOMA	0	0	0	0	1	0.2169
BONE, FEMUR	M-OSTEOSARCOMA	1	0	0	0	0	1
HARDERIAN GLAND	B-ADENOMA	1	3	3	4	4	0.1843
HARDERIAN GLAND	M-CARCINOMA	1	2	0	1	1	0.5467
HEMATO NEOPLASIA	M-SARCOMA, HISTIOCYTIC	1	1	1	0	0	0.9086
HEMATO NEOPLASIA	M-MALIGNANT FIBROUS HISTIOCY	0	0	0	1	0	0.4093
HEMATO NEOPLASIA	M-LYMPHOMA	3	3	4	6	3	0.5054
							0.5781

HEMATO NEOPLASIA	M-PLASMACYTOMA	0	0	1	0	0	0.1951
KIDNEY	B-ADENOMA, TUBULAR CELL	0	0	0	0	1	0.1267
KIDNEY	M-CARCINOMA, TUBULAR CELL	0	0	0	1	1	0.3219
LIVER	M-CARCINOMA, HEPATOCELLULAR	7	9	13	12	11	0.8436
LIVER	B-ADENOMA, HEPATOCELLULAR	5	5	1	5	2	1
LIVER	M-CHOLANGIOCARCINOMA	1	0	0	0	0	0.9198
LUNG	B-ADENOMA, BRONCHIOLOALVEOLA	2	3	2	4	0	0.1846
LUNG	M-CARCINOMA, BRONCHIOLOALVEO	4	7	3	1	8	0.7368
NASAL TURBINATE	B-ODONTOMA	0	1	0	0	0	0.7958
NASAL TURBINATE	M-OSTEOSARCOMA	0	1	0	0	0	0.3927
NASAL TURBINATE	M-SARCOMA, CRANIAL MARROW CA	0	0	0	1	0	0.5
SUBCUTANEOUS TIS	M-RHABDOMYOSARCOMA	0	0	0	1	0	1
SUBCUTANEOUS TIS	M-FIBROSARCOMA, BIOMEDIC IMP	1	0	0	0	0	0.4
SUBCUTANEOUS TIS	B-FIBROMA	0	0	0	1	0	0.2212
STOMACH, GL	M-LEIOMYOSARCOMA	0	0	0	0	1	0.6118
SEMINAL VESICLE	M-LEIOMYOSARCOMA	0	0	1	0	0	0.1225
TESTIS	B-INTERSTITIAL CELL TUMOR	0	0	0	1	1	0.9377
THYROID	B-FOLLICULAR CELL ADENOMA	3	1	3	2	0	0.9268
THYROID	M-FOLLICULAR CELL CARCINOMA	1	1	2	0	0	0.1347
VASC NEOPLASIA	M-HEMANGIOSARCOMA	4	3	4	4	3	0.7778
VASC NEOPLASIA	B-HEMANGIOMA	0	1	0	0	0	

Table A.4: Report on Trend Test –Female Mice

Organ Name	Tumor Name	CTRO	LOW	MED	MEDHI	HIGH	P-Value (Exact Method)
ADRENAL, CORTEX	B-ADENOMA	1	1	0	0	0	0.9556
ADRENAL, MEDULLA	B-PHEOCHROMOCYTOMA, COMPLEX	0	1	0	0	0	0.8213
ADRENAL, MEDULLA	M-MALIGNANT PHEOCHROMOCYTOMA	0	1	0	0	0	0.7903
ADRENAL, MEDULLA	B-PHEOCHROMOCYTOMA	0	0	0	1	0	0.3602
DUODENUM	B-POLYP	0	1	0	0	0	0.7872
BONE, FEMUR	M-OSTEOSARCOMA	0	1	0	0	1	0.2761
HARDERIAN GLAND	B-ADENOMA	2	2	2	1	5	0.0641
HARDERIAN GLAND	M-CARCINOMA	0	0	1	2	2	0.075
HEMATO NEOPLASIA	M-LEUKEMIA, GRANULOCYTIC	1	0	1	0	0	0.8439
HEMATO NEOPLASIA	M-SARCOMA, HISTIOCYTIC	1	0	1	1	0	0.7414

HEMATO NEOPLASIA	M-MALIGNANT FIBROUS HISTIOCY	0	0	1	0	0	0.5957
HEMATO NEOPLASIA	M-LYMPHOMA	8	14	19	9	11	0.5611
HEMATO NEOPLASIA	M-LYMPHOMA, HISTIOCYTIC	1	1	0	1	0	0.7445
HEMATO NEOPLASIA	M-LEUKEMIA	1	1	0	1	1	0.3617
KIDNEY	M-MESENCHYMAL TUMOR	1	0	0	0	0	1
LIVER	M-CARCINOMA, HEPATOCELLULAR	1	3	3	3	3	0.3096
LIVER	B-ADENOMA, HEPATOCELLULAR	3	2	1	4	4	0.1886
LUNG	B-ADENOMA, BRONCHIOLOALVEOLA	0	1	3	2	1	0.4572
LUNG	M-CARCINOMA, BRONCHIOLOALVEO	1	2	1	2	1	0.5215
MAMMARY, FEMALE	B-ADENOMA	1	0	0	0	0	1
MAMMARY, FEMALE	M-CARCINOMA	0	0	0	0	1	0.2117
MAMMARY, FEMALE	B-FIBROADENOMA	0	1	1	0	0	0.7122
NASAL TURBINATE	M-OSTEOSARCOMA	0	1	0	0	0	0.799
OVARY	B-CYSTADENOMA	1	2	2	1	0	0.9011
PANCREAS	B-ADENOMA, ISLET CELL	1	0	0	0	0	1
CAVITY, ABDOM	M-FIBROSARCOMA	0	0	0	0	1	0.3077
PITUITARY	B-ADENOMA	13	14	10	13	15	0.2704
SKIN	M-BASAL CELL CARCINOMA	1	0	0	0	0	1
SUBCUTANEOUS TIS	M-FIBROSARCOMA	1	0	1	1	1	0.417
SUBCUTANEOUS TIS	M-OSTEOSARCOMA	0	0	0	1	0	0.5
SUBCUTANEOUS TIS	M-FIBROSARCOMA, BIOMEDIC IMP	0	1	0	0	1	0.375
SUBCUTANEOUS TIS	M-SCHWANNOMA, MALIGNANT	1	0	0	0	0	1
SKIN, OTHER	M-SQUAMOUS CELL CARCINOMA	0	0	1	0	0	0.6047
STOMACH, NONGL	B-SQUAMOUS CELL PAPILLOMA	0	0	0	1	0	0.3617

Table A.4 (continued): Report on Trend Test – Female Mice

Organ Name	Tumor Name	CTR0	LOW	MED	MEDHI	HIGH	P-Value (Exact Method)
THYROID	B-FOLLICULAR CELL ADENOMA	4	2	4	3	3	0.5094
THYROID	M-FOLLICULAR CELL CARCINOMA	0	0	0	0	1	0.2857
UTERUS	M-CARCINOMA	2	0	0	1	0	0.7651
UTERUS	B-ENDOMETRIAL STROMAL POLYP	1	0	2	2	2	0.1868
UTERUS	B-ADENOMATOID POLYP	0	0	1	0	0	0.5957
UTERUS	M-OSTEOSARCOMA	0	0	0	0	1	0.1862
UTERUS	M-ENDOMETRIAL STROMAL SARCOM	0	0	0	0	1	0.1862
UTERUS	B-LEIOMYOMA	0	0	1	0	0	0.5957
VASC NEOPLASIA	M-HEMANGIOSARCOMA	1	1	3	3	3	0.237
VASC NEOPLASIA	B-HEMANGIOMA	0	0	1	0	0	0.7778

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Figure 2a: Kaplan-Meier Survival Functions for Male Rats

(NDA 21908)

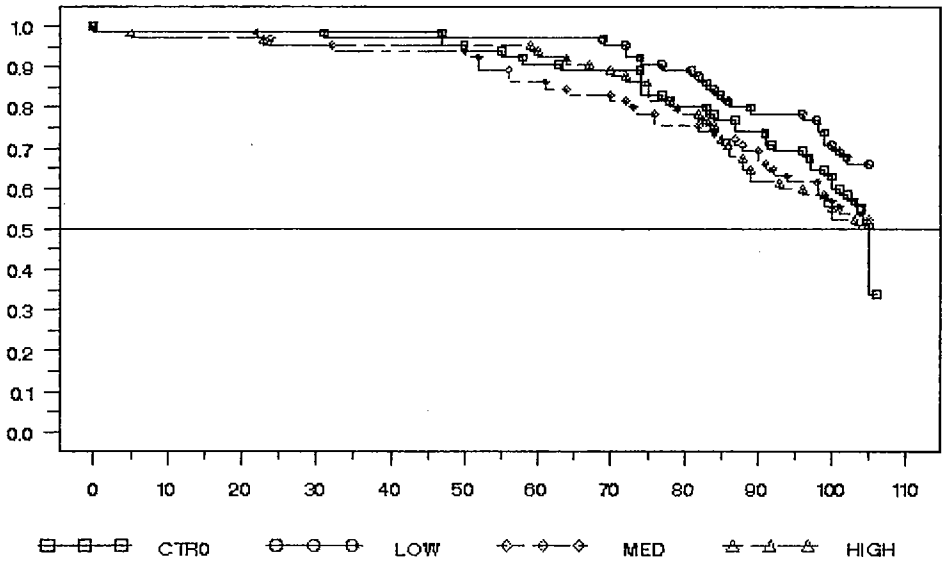

Figure 2b: Kaplan-Meier Survival Functions for Female Rats

(NDA 21908)

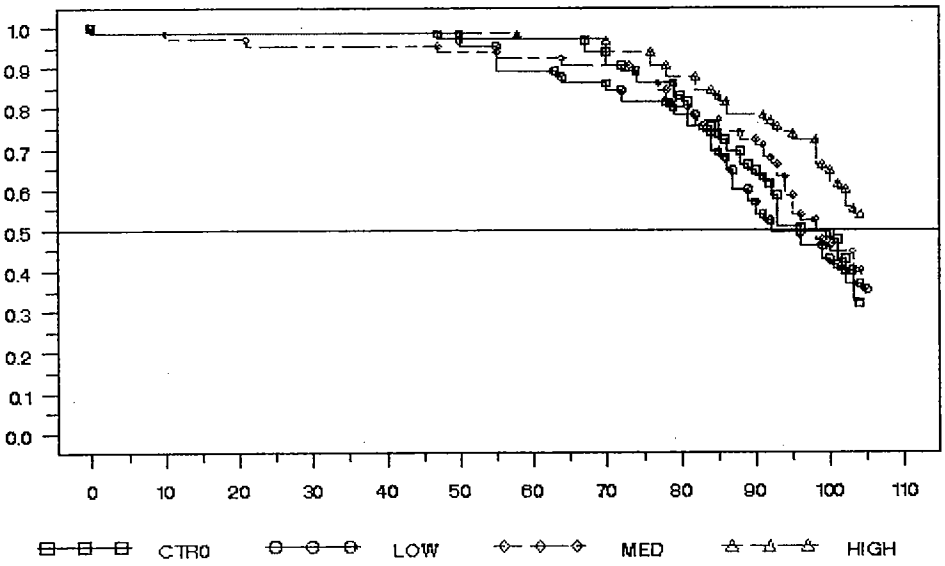

Table A.5: Analysis of Dose-Mortality Trend

Table A.5: Species: Rat, Sex: Male

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	2.7867	0.2482	3.1782	0.2041
Dose-Mortality Trend	1.8764	0.1707	1.8635	0.1722
Homogeneity	4.6632	0.1982	5.0417	0.1688

Table A.6:Analysis of Dose-Mortality Trend

Species: Rat, Sex: Female

	Method			
	Cox		Kruskal-Wallis	
	Statistics	P-Value	Statistics	P-Value
Time-Adjusted Trend Test				
Depart from Trend	0.0593	0.9708	0.3164	0.8537
Dose-Mortality Trend	7.1791	0.0074	7.5192	0.0061
Homogeneity	7.2384	00.0647	7.8357	0.0495

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Table A.7: Report on Trend Test – Male Rats

Organ Name	Tumor Name	CTRL	LOW	MED	HIGH	P-Value (Exact Method)
ADRENAL, MEDULLA	B-PHEOCHROMOCYTOMA	9	14	7	6	0.8781
ADRENAL,	M-MALIGNANT	2	3	2	2	0.4991

MEDULLA	PHEOCHROMOCYTOMA					
BRAIN	M-ASTROCYTOMA	1	1	1	2	0.2214
BRAIN	M-GRANULAR CELL TUMOR	0	1	1	0	0.5937
BRAIN	M-OLIGODENDROGLIOMA	0	1	0	0	0.7461
CECUM	B-LEIOMYOMA	0	1	0	1	0.2882
HEAD, CORONAL	M-OSTEOSARCOMA	0	0	0	1	0.6667
HEMATO NEOPLASIA	M-LYMPHOMA	0	0	0	1	0.2338
HEMATO NEOPLASIA	M-LEUKEMIA, GRANULOCYTIC	1	0	0	0	1
HEMATO NEOPLASIA	M-SARCOMA, HISTIOCYTIC	6	1	4	10	0.0091
KIDNEY	B-LIPOMA	1	0	0	0	1
LIVER	B-ADENOMA, HEPATOCELLULAR	1	1	0	3	0.1109
LIVER	M-CARCINOMA, HEPATOCELLULAR	1	3	1	2	0.375
LN, MESENTERIC	B-HEMANGIOMA	1	0	0	0	1
LN, MESENTERIC	M-HEMANGIOSARCOMA	0	3	0	0	0.8671
NASAL TURBINATE	B-ODONTOMA	1	0	1	0	0.7095
NASAL TURBINATE	M-FIBROSARCOMA	0	0	1	0	0.4595
PANCREAS	M-CARCINOMA, ACINAR CELL	0	1	0	2	0.1003
PANCREAS	B-ADENOMA, ISLET CELL	4	3	1	1	0.9064
PANCREAS	B-ADENOMA, ACINAR CELL	0	1	1	0	0.5937
PANCREAS	M-CARCINOMA, ISLET CELL	0	1	1	0	0.5937
CAVITY, ABDOM	M-OSTEOSARCOMA	0	1	0	0	0.8
CAVITY, ABDOM	B-LIPOMA	0	0	0	2	0.0952
CAVITY, ABDOM	M-FIBROSARCOMA	0	0	0	1	0.375
PITUITARY	B-ADENOMA	26	28	29	23	0.7085
PARATHYROID	B-ADENOMA	0	0	1	0	0.4545
SPLEEN	M-HEMANGIOSARCOMA	0	0	1	0	0.6129
SUBCUTANEOUS TIS	M-SQUAMOUS CELL CARCINOMA	0	0	1	0	0.5714
SUBCUTANEOUS TIS	B-FIBROMA	2	0	0	3	0.339
SUBCUTANEOUS TIS	M-FIBROSARCOMA	0	1	2	0	0.8482
SKIN, OTHER	M-SARCOMA, NOS	0	1	0	0	0.723
SKIN, OTHER	B-KERATOACANTHOMA	4	3	2	3	0.4874
SKIN, OTHER	M-MYXOSARCOMA	0	1	0	0	0.7197
SKIN, OTHER	B-FIBROMA	2	0	0	0	1
SKIN, OTHER	B-SQUAMOUS CELL PAPILLOMA	0	0	0	1	0.202
SKIN, OTHER	M-SQUAMOUS CELL CARCINOMA	0	0	0	1	0.202
SKIN, OTHER	M-BASAL CELL CARCINOMA	0	1	0	0	0.7374
SKIN, OTHER	B-TRICHOEPITHELIOMA	0	0	1	0	0.404
SKIN, OTHER	M-AMELANOTIC MELANOMA	0	0	1	0	0.45

Table A.7 (continued): Report on Trend Test – Male Rats

Organ Name	Tumor Name	CTR0	LOW	MED	HIGH	P-Value (Exact Method)
ADRENAL,	B-PHEOCHROMOCYTOMA	9	14	7	6	0.8781

MEDULLA						
STOMACH, NONGL	B-SQUAMOUS CELL PAPILLOMA	1	1	5	6	0.0232
STOMACH, NONGL	M-SQUAMOUS CELL CARCINOMA	1	0	1	1	0.3343
CAVITY, THORACIC	B-FIBROMA	0	1	0	0	1
CAVITY, THORACIC	M-OSTEOSARCOMA	1	0	0	0	1
TESTIS	B-INTERSTITIAL CELL TUMOR	2	4	1	10	0.0006
THYMUS	M-THYMOMA	1	0	0	1	0.4434
THYROID	M-"C" CELL CARCINOMA	1	4	1	0	0.9384
THYROID	B-FOLLICULAR CELL ADENOMA	2	5	0	2	0.7379
THYROID	M-FOLLICULAR CELL CARCINOMA	0	1	0	0	0.7568
THYROID	B-"C" CELL ADENOMA	5	7	5	6	0.3605
ZYMBAL'S GLAND	M-SQUAMOUS CELL CARCINOMA	0	0	1	2	0.0562

Table A.8: Report on Trend Test – Female Rats

Organ Name	Tumor Name	CTR0	LOW	MED	HIGH	P-Value (Exact Method)
ADRENAL, CORTEX	B-ADENOMA	3	0	0	0	1
ADRENAL, MEDULLA	B-PHEOCHROMOCYTOMA	1	1	5	0	0.9148

ADRENAL, MEDULLA	M-MALIGNANT PHEOCHROMOCYTOMA	0	0	0	1	0.3302
BRAIN	M-ASTROCYTOMA	1	0	0	2	0.1003
BRAIN	M-GRANULAR CELL TUMOR	0	1	0	0	0.7606
CERVIX	B-GRANULAR CELL TUMOR	0	1	0	0	0.8889
HEAD, CORONAL	M-OSTEOSARCOMA	0	0	1	0	1
HEMATO NEOPLASIA	M-LYMPHOMA	0	0	1	0	0.4961
HEMATO NEOPLASIA	M-SARCOMA, HISTIOCYTIC	0	1	1	4	0.0277
HEART	M-ENDOCARDIAL SCHWANNOMA	0	0	1	0	0.5401
KIDNEY	M-NEPHROBLASTOMA	0	0	1	0	0.4981
LIVER	B-ADENOMA, HEPATOCELLULAR	0	0	1	5	0.031
MAMMARY, FEMALE	B-FIBROADENOMA	14	18	14	15	0.6502
MAMMARY, FEMALE	M-CARCINOMA, MULTIPLE	1	2	1	0	0.9182
MAMMARY, FEMALE	M-FIBROSARCOMA	0	0	0	1	0.2661
MAMMARY, FEMALE	B-FIBROADENOMA, MULTIPLE	2	1	4	4	0.1979
MAMMARY, FEMALE	B-FIBROMA	0	0	0	1	0.2388
MAMMARY, FEMALE	M-CARCINOSARCOMA	0	1	0	0	0.7461
MAMMARY, FEMALE	M-CARCINOMA	12	6	5	4	0.967
OVARY	M-GRANULOSA/THECA CELL TUMOR	1	0	0	3	0.0711
OVARY	B-TUBULAR ADENOMA, SERTOLIFO	0	0	0	1	0.3302
OVARY	B-GRANULOSA/THECA CELL TUMOR	0	0	1	0	0.5755
PANCREAS	M-CARCINOMA, ACINAR CELL	0	0	0	1	0.2579
PANCREAS	B-ADENOMA, ISLET CELL	3	1	0	0	0.9968
PANCREAS	M-CARCINOMA, ISLET CELL	2	0	0	0	1
PITUITARY	B-ADENOMA	52	47	47	37	0.9999
PARATHYROID	B-ADENOMA	0	0	0	1	0.3204
SUBCUTANEOUS TIS	M-SQUAMOUS CELL CARCINOMA	0	0	1	0	0.6
SUBCUTANEOUS TIS	M-SCHWANNOMA	0	0	1	0	0.5
SKIN, OTHER	M-HEMANGIOSARCOMA	1	0	0	0	1
SKIN, OTHER	M-SCHWANNOMA	0	0	0	1	0.4068
SKIN, OTHER	B-NEUROFIBROMA	0	1	0	0	0.7727
STOMACH, NONGL	B-SQUAMOUS CELL PAPILLOMA	0	0	1	0	0.5373
STOMACH, NONGL	M-SQUAMOUS CELL CARCINOMA	0	1	0	1	0.3734
CAVITY, THORACIC	B-LIPOMA	0	1	0	0	1
CAVITY, THORACIC	M-MALIGNANT HIBERNOMA	0	0	1	0	0.6667
CAVITY, THORACIC	M-CHORDOMA	0	0	1	0	0.6667
THYROID	M-"C" CELL CARCINOMA	1	1	0	1	0.6117
THYROID	B-FOLLICULAR CELL ADENOMA	1	1	0	2	0.3321
THYROID	M-FOLLICULAR CELL CARCINOMA	0	1	0	0	0.8019
THYROID	B-"C" CELL ADENOMA	3	6	11	4	0.7316

URINARY BLADDER	M-LEIOMYOSARCOMA	1	0	0	0	1
URINARY BLADDER	B-TRANSITNL. CELL PAPILLOMA	0	0	1	0	0.5769
URETHRA	B-HEMANGIOMA	0	1	0	0	0.5
UTERUS	B-ENDOMETRIAL STROMAL POLYP	2	0	1	4	0.0842
UTERUS	M-CARCINOMA	0	0	0	1	0.3171
UTERUS	M-SCHWANNOMA	0	1	0	0	0.8019
VAGINA	M-LEIOMYOSARCOMA	0	0	1	1	0.2331
VAGINA	B-GRANULAR CELL TUMOR	1	0	0	0	1
ZYMBAL'S GLAND	B-ADENOMA	1	0	0	0	1

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/s/

Mushfiqur Rashid
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Karl Lin
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Concur with review