

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

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STATISTICAL REVIEW(S)



U.S. Department of Health and Human Services
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Office of Biostatistics

STATISTICAL REVIEW AND EVALUATION

CLINICAL STUDIES

NDA/Serial Number: 20-497/N000

Drug Name: Alinia™ (nitazoxnide) 500 mg tablets

Indication(s): Treatment of Diarrhea caused by *Giardia lamblia* in adults and adolescent patients greater than 11 years of age.

Applicant: Romark Laboratories, L.C.

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1. EXECUTIVE SUMMARY

1.1 Conclusions and Recommendations

This resubmission of NDA 21-497 contains one controlled clinical study for the treatment of diarrhea caused by *Giardia lamblia* in adults and adolescents (> 11 years of age).

The efficacy of NTZ tablets for the treatment of diarrhea caused by *Giardia lamblia* in adults and adolescents (≥ 12 years of age) was studied in this application, in a randomized, double-blind, placebo-controlled study conducted in one center in Egypt and one center in Peru.

This study demonstrated superior efficacy of NTZ tablets over placebo in terms of 7-10 day clinical response rate (defined as 'no symptoms, no watery stools and no more than 2 soft stools with no hematochezia within the past 24 hours' or 'no symptoms and no unformed stools within the past 48 hours'). The 95% confidence interval for the difference in clinical response rates for the tablet and suspension was (-14%, 17%).

Parasitological response rate at day 7-10 for NTZ tablets, although statistically significantly better than placebo, correlated weakly to the clinical response rate as measured by kappa coefficient (kappa = 0.196) and the difference in cyst counts in concentrated stool samples from baseline to day 7-10 was not statistically significantly different from placebo.

Parasitological response at day 14-17, again although statistically significantly better than placebo in clinical responders at day 7-10 was not accompanied by clinical evaluation and showed some worsening in terms of cyst counts in concentrated stool samples for NTZ tablets—especially for the center in Peru.. These results are inconclusive due to small sample sizes.

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1.2 Brief Overview of Clinical Studies

Nitazoxanide tablets and suspension had been previously studied for the treatment of diarrhea caused by *Giardia lamblia* in two controlled clinical studies (RM-NTZ 98-001 and RM-NTZ 99-010) that were designed and conducted using the proposed three-day treatment regimen in the previous submission of NDA 21-497 (and NDA 21-498 for suspension). The results of these studies are briefly discussed in Section 1.3. The current resubmission of NDA 21-497 contains one randomized, placebo-controlled, double-blind study (RM01-3011) conducted at two centers one in Peru and one in Egypt. The following table provides a brief overview of all three studies.

Table 1: Controlled clinical Trials in NDA 21497 for diarrhea caused by *Giardia lamblia*

| | | | | | | |
|------------------|---------------|--|---|----|-------|--------|
| RM-NTZ 98-001 | Egypt | Randomized double-blind Placebo- controlled | NTZ tablet: 500 mg bid | 47 | 12-65 | 3 days |
| | | | Placebo:bid | 44 | 12-65 | 3 days |
| RM-NTZ 99-010 | Peru | Randomized single-blind Active- controlled | NTZ suspension: 100 mg bid | 14 | 2-3 | 3 days |
| | | | 200 mg bid | 41 | 4-11 | 5 days |
| | | | Metronidazole 125 mg bid | 29 | 2-3 | |
| | | | 250 mg bid | 26 | 4-11 | |
| RM01- 3011 | Egypt Peru | Randomized double-blind Placebo- controlled | NTZ tablet: 500 mg bid | 54 | 12-55 | 3 days |
| | | | NTZ suspension: 25 ml of 20mg/ml bid | 54 | 12-51 | 3 days |
| | | | Placebo:bid | 27 | 12-34 | 3 days |

1.3 Statistical Issues and Findings

In the previous submission of NDA 21-497 (and NDA 21-498), the sponsor had submitted two randomized, single center, controlled studies (RM-NTZ 98-001 and RM-NTZ 99-010) for the treatment of diarrhea caused by *Giardia lamblia*. Single center, single race nature of these two studies seriously compromised the generalizability of the results to the general population.

Study RM-NTZ 98-001 was a placebo-controlled study conducted in adults and adolescents for NTZ tablet in Egypt. In this study, when patients infected with only *Giardia lamblia* infection were isolated, the sample size was reduced to 18 patients, of whom, only 8 were randomized to NTZ (just one patient out of 8 was female). These sample sizes were very small. Also, kappa coefficient for the NTZ arm in the group of patients with giardiasis was negative (-0.283), compromising the meaningfulness of the parasitological endpoint.

Study RM-NTZ 99-010 was active (metronidazole) controlled study in children 2 to 11 years of age for NTZ suspension in Peru. In this study, the non-inferiority of NTZ compared to metronidazole with a margin of 20% was demonstrated with 95% confidence in terms of the clinical response rates (85% for NTZ versus 80% for Metronidazole), but not in terms of the parasitological response rates. The kappa coefficients for both NTZ and metronidazole arms were positive and comparable (0.276 for NTZ, 0.227 for Metronidazole). All subgroup/special protocol analyses in this study, such as ones based on gender, subgroup of patients with only *Giardia lamblia* infection, “per protocol” population, all showed results consistent with the Intent-to-Treat population. Thus, the data in children’s population provided adequate evidence of efficacy of NTZ suspension for the treatment of Diarrhea caused by *Giardia lamblia*,

The study reviewed in this document was the third study of the efficacy of tablets for the

treatment of diarrhea caused by *Giardia lamblia*. This study was randomized, double-blind, placebo-controlled study conducted in one center in Egypt and one center in Peru in adults and adolescents (age > 11 years). This study showed that 7-10 day clinical response rate (defined as no symptoms, no watery stools and no more than 2 soft stools with no hematochezia within the past 24 hours' or 'no symptoms and no unformed stools within the past 48 hours) for NTZ tablets (85%) was statistically significantly better than placebo (44%) (p-value = 0.0002). The 95% confidence interval for the difference in clinical response rates for the tablet and suspension was (-14%, 17%). Parasitological response rate for NTZ tablets, although statistically significant, correlated weakly to the clinical response rate as measured by kappa coefficient (kappa = 0.196) and the difference in cyst counts in concentrated stool samples from baseline to day 7-10 was not statistically significantly different from placebo. Parasitological response at day 14-17, again although statistically significantly better than placebo in clinical responders at day 7-10 was not accompanied by clinical evaluation and showed some worsening in terms of cyst counts in concentrated stool samples for NTZ tablets, especially at the center in Peru. These results are inconclusive due to small sample sizes.

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2. INTRODUCTION

2.1 Overview

Nitazoxanide was originally synthesized in the early 1970s, but was not fully developed at that time. Romark Laboratories re-initiated the development of nitazoxanide in the 1990s for treating infections caused by a broad spectrum of parasites that infect intestinal tracts of humans including *Cryptosporidium parvum* and *Giardia lamblia* infections. The first submission of NDA 21-497 (and NDA 21-498 for suspension), included five controlled clinical studies that were designed and conducted using the proposed three-day treatment regimen to demonstrate the efficacy of nitazoxanide in treating diarrhea caused by *C. parvum* and *Giardia lamblia*. Both of these diseases are considered orphan diseases in the United States. Two of these studies contained information regarding efficacy of nitazoxanide for the treatment of diarrhea caused by *Giardia lamblia*. The study in adults and adolescents (> 11 years of age) did not provide adequate evidence of efficacy of NTZ tablets for the treatment of diarrhea caused by *Giardia lamblia*.

The study in children (2-11 years of age) provided adequate evidence of efficacy of NTZ suspension for the treatment of Diarrhea caused by *Giardia lamblia*,

This document contains the review of one multicenter, double-blind, placebo-controlled study (RM01-3011) of nitazoxanide 500 mg tablets in the treatment of diarrhea caused by *Giardia Lamblia* in Adults and adolescents (>11 years of age).

2.2 Data Sources

The data sets for the study RM01-3011 were submitted electronically at the following location:

\\Cdsub1\N21497

The file name used was N_000\2004-01-28\CRT\DATASETS\RM013011.

This reviewer found the efficacy data sets to be well organized and of good quality. Also this reviewer did not find notable discrepancies between the results given in the text of the sponsor's study report and those obtained using the submitted data sets.

3. STATISTICAL EVALUATION

3.1 Evaluation of Efficacy

3.1.1 Study Design and Endpoints

The study RM01-3011 was a multi-center, double-blind, placebo-controlled study conducted at two centers one in Peru and one in Egypt.

Main criteria for inclusion were as follows: at least 12 years of age, diarrhea (≥ 3 bowel movements/day) with one or more anergic symptoms (e.g. abdominal pain or cramps, nausea, vomiting, tenesmus or malabsorption) and cysts of *G. lamblia* in a stool specimen collected within 7 days prior to enrollment.

Upon enrollment, patients were randomized to one of the three treatment groups: NTZ 500 mg tablet bid with food, 25 ml of NTZ 100mg/5mL suspension bid with food, or placebo bid with food. Patients received treatment for 3 consecutive days.

Reviewer's Comment: It appears that the randomization was stratified by center and the randomization ratio was 2:2:1 (tablet:suspension:placebo). Both of these issues were not mentioned in the sponsor's study report as well as the protocol.

At the screening visit as well as at baseline visit, stool samples were subjected to microscopic examination (concentrated and unconcentrated) for the detection of cysts of *G. lamblia*. Stool samples were subjected to either an immunofluorescence assay or an enzyme immunoassay for the detection of *G. lamblia* and *C. parvum*. Patients were considered positive for cysts of *G. lamblia* if any of the tests for *G. lamblia* were positive.

Patients who were not positive for cysts of *G. lamblia* at baseline and patients with bacterial causes of diarrhea were to be excluded from the analysis of efficacy.

Each patient was given a diary to record administration of the medication, adverse events and information related to the number of stools per day and their consistency.

Patients were evaluated 7 to 10 days after the start of therapy. Two stool samples collected at least 24 hours apart between day 7 and 10 were subjected to microscopic examination (concentrated and unconcentrated) and if possible, to an immunofluorescence assay for the detection of cysts of *G. lamblia*. Patients were considered positive for cysts of *G. lamblia* if any of these tests were positive.

All clinical responders at day 7-10 evaluation were to return between days 14 and 17 and submit one stool sample for examination for *G. lamblia* cysts or trophozoites.

On day 7-10, patients were assigned **clinical response** (well, continuing illness, clinical

failure) and **parasitological response** (eradication or persistence) based on two stool samples. For the clinical endpoint, 'well' response was defined as 'no symptoms, no watery stools and no more than 2 soft stools with no hematochezia within the past 24 hours' or 'no symptoms and no unformed stools within the past 48 hours.' For the parasitological endpoint, 'Eradication' was defined as 'no cysts of the parasite observed in either of the 2 post-treatment parasitological examinations'. In the protocol, clinical response was stated as primary endpoint and parasitological response was stated as secondary endpoint. Time from initiation of treatment to passage of last unformed stool (as reported by the patient in a diary) was stated as secondary efficacy endpoint in the study protocol. However the data required for the analysis of this endpoint was not collected and therefore analysis could not be performed.

Assuming a clinical response rate of 85% for nitazoxanide suspension and that the expected response rate for the nitazoxanide tablets is equal to that of the nitazoxanide suspension and using a two-group large-sample normal approximation test of proportions with a one-sided 0.025 significance level, a sample size of 54 patients in each group was deemed sufficiently powerful (82%) to reject the null hypothesis that nitazoxanide tablets and nitazoxanide suspension are not equivalent (the response rate for nitazoxanide tablets is inferior to that of nitazoxanide suspension by 20% or more) in favor of the alternative hypothesis that the proportions in the two groups are equivalent. A Fisher's exact test with a 0.05 two-sided significance level had 97% power to detect the difference between an expected response rate of 85% for each active treatment group and an expected response rate of 40% for the placebo group when the sample sizes were 54 for each of the active treatment groups and 27 for the placebo group.

3.1.2 Planned Statistical Analyses

Data for a modified intent-to-treat population was to be used for the primary efficacy analysis. The modified intent-to-treat population will consist of all patients randomized to the study excluding:

- patients who do not have *Giardia* trophozoites or cysts in their stool at baseline and
- have other identified causes of diarrhea at baseline (e.g., pathogenic bacteria, *C. parvum*, *E. histolytica*).

A secondary analysis of all patients randomized to the study would also be performed. In addition, in the event that there are a significant number of protocol deviations, consideration will be given to analysis of a subset of patients who complete the study according to the protocol.

The primary efficacy analysis was defined by the protocol as a comparison of the proportional clinical response for the nitazoxanide tablet group to that of the placebo group. Planned secondary efficacy analyses included: (1) comparison of proportional clinical response rates for the nitazoxanide tablets and nitazoxanide suspension, (2) comparison of proportional clinical response rates for the nitazoxanide suspension and placebo, (3) comparison by treatment group of the median time from initiation of treatment to passage

of last unformed stool, (4) comparison of parasitological response rates for the nitazoxanide tablets and placebo, (5) comparison of the proportional parasitological response rates for the nitazoxanide tablets and suspension, (6) comparison of proportional parasitological response rates for the nitazoxanide suspension and placebo, (7) inpatient correlation of parasitological outcome with clinical outcome for each treatment group, (8) comparison of potential food effect on efficacy for active treatment groups, and (9) comparison by treatment group of the results of day 14-17 stool examinations for clinical responders.

Proportional clinical and parasitological response rates, inpatient correlation of parasitological outcome with clinical outcome, the potential effect of food on efficacy, and the results of stool examinations at day 14-17 for clinical responders were to be compared using Fisher's Exact tests (two-sided). Two-sided 95% confidence intervals were to be calculated for the differences in the proportional clinical and parasitological response rates using the preferred method described by Newcombe (1998) with correction for continuity. The comparison by treatment group of the median time from the beginning of treatment to the passage of the last unformed stool was to be conducted using Kaplan-Meier survival analysis.

Adverse events were summarized for each treatment group, and the proportions of patients in each group experiencing adverse events were compared using chi-square tests.

3.1.3 Patient Disposition

The following table reports the patient disposition for the two studies by the center.

Table 2: Patient Disposition

| | Egypt | Peru | Total |
|------------------|--------------|---|--------------|
| Screened | 593 | 4278 | 4871 |
| Enrolled | 45 | 90 | 135 |
| Completed | 45 | 88 | 133 |
| Withdrawn | 0 | 2 1-adverse event 1-loss to follow-up | 2 |

Of the 45 patients enrolled in Egypt, 18 were randomized to NTZ tablets, 18 to NTZ suspension and 9 to placebo. Of the 90 patients enrolled in Peru, 36 were randomized to NTZ tablets, 36 to NTZ suspension and 18 to placebo. The patient who withdrew due to adverse event was assigned to NTZ tablets group and the patient lost to follow-up was assigned to NTZ suspension group. These two patients were treated as failures in the efficacy analysis.

All patients randomized to the study were included in the efficacy analysis. The modified intent to treat population was identical to the population of all randomized patients.

3.1.4 Demographic and Baseline Characteristics

A summary of demographic data and disease-related characteristics is presented in the following table. These were generally comparable across the treatment groups.

Table 3 : Demographic and Disease-Related Characteristics

| Characteristics | NTZ tablets (N= 54) | NTZ suspension (N=54) | Placebo (N=27) |
|---|---------------------|-----------------------|----------------|
| Race (count) | | | |
| Hispanic | 36 (67%) | 36 (67%) | 18 (67%) |
| Caucasian | 18 (33%) | 18 (33%) | 9 (33%) |
| Gender (count) | | | |
| Male | 34 (63%) | 34 (63%) | 23 (85%) |
| Female | 20 (37%) | 20 (37%) | 4 (15%) |
| Age (years) | | | |
| Mean (s.d.) | 19.98 (10.29) | 20.13 (10.19) | 18.87 (7.10) |
| Range | 12-55 | 12-51 | 12-34 |
| Weight (kgs) | | | |
| Mean (s.d.) | 53.34 (16.16) | 51.80 (13.87) | 53.78 (16.86) |
| Range | 25-100 | 25-81 | 28-80 |
| Stool Frequency (count) | | | |
| 1-2/day | 1 (2 %) | 1 (2%) | 1 (37%) |
| 3-4/day | 51 (94%) | 50 (92%) | 26 (63%) |
| 5-10/day | 2 (4 %) | 3 (6%) | 0 |
| Stool Consistency (count) | | | |
| Liquid | 12 (22%) | 9 (16%) | 5 (9%) |
| Soft | 42 (78%) | 43 (80%) | 22 (91%) |
| Formed | 0 | 2 (4 %) | 0 |
| Duration of Diarrhea (days) | | | |
| Median | 5.5 | 6 | 6 |
| Mean (s.d.) | 5.74 (2.5) | 6.41* (3.52) | 6.59 (2.95) |
| Range | 1-14 | 2-27* | 2-18 |
| Giardia Cyst quantification (concentrated stool) | | | |
| Median | 3.5 | 3 | 5 |
| Mean (s.d.) | 6.2 (11.7) | 5.3 (9) | 6.5 (8.1) |
| Range | 0-80 | 0-60 | 0-38 |
| No. of patients with >10 cysts | 6 | 5 | 5 |

*: Excludes one outlier with diarrhea for more than 5 years.

3.1.5 Sponsor's Efficacy Results and Conclusions

All patients randomized to the study were included in the efficacy analyses. The following table shows sponsor's analyses for the clinical (primary) and parasitological response.

Table 4: Sponsor's Efficacy Results

| | NTZ tablets | NTZ Suspension | Placebo | 95% CI on the difference (tablet- suspension) |
|---|------------------------|---------------------------|------------------|--|
| Clinical Response: day 7-10 (Well) | 46/54 (85.2%) | 45/54 (83.3%) | 12/27 (44.4%) | (-13.5%, 17.1%)** |
| p-value vs. placebo | 0.0002* | 0.0006** | | |
| Parasite Response : day 7-10 (Eradication) | 30/54 (55.6%) | 26/54 (48.2%) | 5/27 (18.55) | (-12.4%, 26.4%)** |
| p-value vs. placebo | 0.0019** | 0.0146** | | |
| Parasite Response[#] : day 14-17 (Negative) | 22/45 (48.9%) | 24/43 (55.8%) | 3/12 (25%) | (-15.3%, 26.3%)** |
| p-value vs. placebo | 0.1953** | 0.1011** | | |

[#]: for population patients who were clinical responders at day 7-10 visit. One clinical responder in the NTZ tablet group and two in the NTZ suspension group did not submit a day 14-17 stool sample.

*: primary comparison. **: secondary comparison

The sponsor stated that planned secondary analyses of time to last unformed stool and potential food effect were not performed as the data needed for these analyses were either not collected or were too small in sample size.

The sponsor presented the intra-patient correlation between clinical and parasitological response by conducting Fisher's exact test. Also the results of chi-square test for stool examinations at day 14-17 are compared by treatment group for clinical responders were presented.

In addition, the sponsor presented subgroup analyses by center and several post-hoc exploratory analyses to explain the differences between the two centers in the study.

The sponsor conclusions were as follows:

This study demonstrated the efficacy of nitazoxanide tablets compared to placebo tablets in treating diarrhea caused by *Giardia lamblia*. The clinical response rates observed during this study were comparable to those observed during other controlled studies of the nitazoxanide tablets in adults and the nitazoxanide suspension in pediatric patients. Furthermore, the study demonstrated the efficacy of the nitazoxanide tablets is equivalent (not inferior) to that of the nitazoxanide suspension in this indication. Importantly, the study demonstrated efficacy of the nitazoxanide tablets and nitazoxanide suspension in patients from both hyperendemic and non-hyperendemic areas. Patients from Peru where *Giardia* is hyperendemic were more likely to have *Giardia* cysts observed in post-treatment stool samples, a factor that may be attributed to either (1) failure to eliminate all of the cysts with a single course of treatment in these patients and/or (2) continuous ingestion of *Giardia* cysts by these patients during the treatment and follow-up period.

3.1.6 Reviewer's Analyses, Efficacy Results and Conclusions

The analyses of clinical and parasitological response for the population of all randomized patients conducted by this reviewer showed results identical to the ones reported in Table 4 and will not be replicated here.

Medical Reviewer identified 16 patients with zero cyst counts in the baseline unconcentrated and concentrated stool samples. For the detail information of these patients, the reader is referred to the clinical review by Joette Meyer, Pharm. D. Results of the sensitivity analyses of clinical and parasitological response excluding these 16 patients are given in the following table. These results are similar to the results in Table 3.

Table 5 : Results of the Sensitivity analyses for day 7-10 visit

| | NTZ tablets | NTZ Suspension | Placebo | 95% CI on the difference (tablet- suspension) |
|--|--------------------|-----------------------|----------------|--|
| Clinical Response (Well) | 39/47 (83%) | 38/47 (80.9%) | 11/25 (44%) | (-14.9%, 19.1%)** |
| p-value vs. placebo | 0.0011* | 0.0029** | | |
| Parasite Response (Eradication) | 23/47 (48.9%) | 20/47 (42.6%) | 4/25 (16%) | (-13.2%, 25.9%)** |
| p-value vs. placebo | 0.0098** | 0.0347** | | |

* : primary comparison. ** : secondary comparison

This reviewer computed kappa coefficients to assess the correlation between the clinical and parasitological response. These are given in the following table.

Table 6 : Correlation between Clinical and Parasitological Response at day 7-10 visit

| | NTZ tablets | NTZ Suspension | Placebo |
|--|--------------------|-----------------------|----------------|
| Clinical Response (Well) | 46/54 (85.2%) | 45/54 (83.3%) | 12/27 (44.4%) |
| Parasite Response (Eradication) | 30/54 (55.6%) | 26/54 (48.2%) | 5/27 (18.55) |
| Kappa | 0.283 | 0.313 | 0.196 |

These results indicate that the correlation between clinical and parasitological endpoints is weak. In a sensitivity analysis excluding those 16 patients with zero cyst count in concentrated and unconcentrated stool, the kappa coefficients were similar to the ones reported in Table 5.

This reviewer also conducted one-way analysis of variance on the cysts counts in concentrated stool on day 0, on the difference between the cysts counts in concentrated stool on day 0 minus day 7 and for day 7 clinical responders, on the difference between the concentrated cyst counts stool on day 14 minus day 7. The results of the F test for day 0 showed no significant difference between the three treatment groups (tablet, suspension and placebo) at baseline (p-value = 0.83). The results of the F test for the difference between the cyst counts day 0 minus day 7 showed no significant difference between the three treatment groups (p-value = 0.73). Also, the results of the F test for the difference between the cyst counts day 14 minus day 7 showed no significant difference between the three treatment groups (p-value = 0.87).

3.2 Evaluation of Safety

There were no deaths reported in this study. There was one serious adverse event in NTZ tablet group that was considered unrelated to the treatment. Sixty-one of the 135 randomized subjects in the study reported at least one adverse event. These events were generally considered mild and many could be considered symptoms of giardiasis. For a more detailed discussion of adverse events and safety of this drug, please refer to the clinical review (of Joette Meyer, PharmD).

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4. FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

The p-values reported in this section are nominal p-values and do not include adjustment for multiplicity. If significant, these p-values do not provide confirmatory evidence, and may only be used for hypotheses generating.

4.1 Gender, Race and Age

The study in this application was conducted in two centers. Each center represented a population from a single race. Therefore race differences if any are confounded with the geographic location differences. The results of analyses by center are given in Section 4.2.

For covariate age, this reviewer conducted logistic regression using the primary efficacy variable as the response variable and treatment, covariate and treatment-by-covariate interaction as the explanatory variables. In this analysis, age-by-treatment interaction was not statistically significant ($p > 0.9$).

In this study, the patient population consisted of 63% of males and 37% of females. However the percent of females in the placebo group was disproportionately lower (15%) consisting of only 4 subjects. Therefore a meaningful assessment of gender effect can not be made using these data.

4.2 Other Special/Subgroup Populations

A subgroup analysis of patients with more than 10 cysts counts in concentrated stool at baseline was performed. There were only 16 patients (6 NTZ tablet, 5 NTZ suspension, 5 placebo) in this subgroup. The clinical response rates were as follow: 100% (6/6) for NTZ tablet, 60% (3/5) for NTZ suspension and 20% (1/5) for placebo. Due to small sample sizes, these results can not be generalized.

As mentioned in the earlier sections, this reviewer also performed analyses of the clinical trial data by center. The results for clinical and parasitological endpoints and their correlations and the results for difference cyst counts in concentrated stool samples from baseline to day 7 are given below.

Table 7: Efficacy Results by Center for day 7-10 visit

| | NTZ tablets | NTZ Suspension | Placebo | 95% CI on the difference (tablet- suspension) |
|--|------------------------|---------------------------|-----------------|--|
| EGYPT | | | | |
| Clinical Response (Well) | 17/18 (94.4%) | 16/18 (88.9%) | 3/9 (33.3%) | (-17%, 28%) |
| p-value vs. placebo | 0.0017 | 0.0061 | | |
| Parasite Response (Eradication) | 17/18 (94.4%) | 15/18 (83.3%) | 2/9 (22.2%) | (-12%, 34%) |
| p-value vs. placebo | 0.0003 | 0.0036 | | |
| Kappa | ~ 0.44 | 0.77 | 0.18 | |
| PERU | | | | |
| Clinical Response (Well) | 29/36 (80.6%) | 29/36 (80.6%) | 9/18 (50%) | (- 20%, 20%) |
| p-value vs. placebo | 0.029 | 0.029 | | |
| Parasite Response (Eradication) | 13/36 (36.1%) | 11/36 (30.6%) | 3/18 (16.7%) | (-16%, 27%) |
| p-value vs. placebo | 0.2087 | 0.3387 | | |
| Kappa | 0.15 | 0.19 | 0.33 | |

In a one-way analysis of variance on the cysts counts in concentrated stool on day 0 and on the difference between the cysts counts in concentrated stool on day 0 minus day 7, the results of the F test for day 0 showed no significant difference between the three treatment groups (tablet, suspension and placebo) at baseline for both centers (p-value =0.32 for Egypt and p-value =0.94 for Peru). Also, the results of the F test for the difference between the cyst counts day 0 minus day 7 also showed no significant difference between the three treatment groups for both centers (p-value = 0.14 for Egypt and p-value = 0.84 for Peru).

These results show that clinical response rates for NTZ tablet as well as suspension, although statistically significantly superior to placebo at both centers, are lower in Peru than in Egypt. Also, at Peru site, the statistical significance of parasitological response is not maintained and the kappa coefficients are very small indicating very weak correlation between clinical and parasitological endpoints. These center differences may be attributed to the fact that Peru is hyperendemic area where as Egypt is not.

The following table (also given in a different format in the sponsor' study report) show the comparison of the two study sites in terms of clinical response on day 7, parasitological response on day 7 and parasitological response on day 14 (follow-up visit) for all three treatment groups.

Table 8: Comparison of study Centers

| | Peru | Egypt | p-value |
|--|-------------|--------------|----------|
| Clinical Response at day 7 | | | |
| NTZ tablet | 29/36 (81%) | 17/18 (94%) | 0.24 |
| NTZ suspension | 29/36 (81%) | 16/18 (89%) | 0.70 |
| Placebo | 9/18 (50%) | 3/9 (33%) | 0.68 |
| Parasite Response at day 7 | | | |
| NTZ tablet | 13/36 (36%) | 17/18 (94%) | < 0.0001 |
| NTZ suspension | 11/36 (31%) | 15/18 (83%) | 0.0004 |
| Placebo | 3/18 (17%) | 2/9 (22%) | 1.0 |
| Parasite Response at day 14[#] | | | |
| NTZ tablet | 6/29 (21%) | 16/16 (100%) | <0.0001 |
| NTZ suspension | 10/27 (37%) | 14/16 (88%) | 0.0016 |
| Placebo | 1/9 (11%) | 2/3 (67%) | 0.13 |

[#] : for population patients who were clinical responders at day 7-10 visit. One clinical responder in the NTZ tablet group and two in the NTZ suspension group did not submit a day 14-17 stool sample.

These results show a statistically significant difference between the two centers in the parasitological response rates at day 7 and day 14 for the NTZ tablets and NTZ suspension groups. The response rates observed in Peru are lower than the ones in Egypt. Again, as suggested by the sponsor, these center differences may be attributed to the fact that Peru is hyperendemic area where as Egypt is not.

5. SUMMARY AND CONCLUSIONS

5.1 Statistical Issues and Collective Evidence

There are no additional statistical and / or technical issues that need to be addressed.

This document contains statistical review of one pivotal study.

In the previous submission of NDA 21-497 (and NDA 21-498 for suspension), the sponsor had submitted two randomized, single center, controlled studies for the treatment of diarrhea caused by *Giardia lamblia*. Single center, single race nature of these two studies seriously compromised the generalizability of the results to the general population.

One was a placebo-controlled study conducted in adults and adolescents for NTZ tablet in Egypt. In this study, when patients infected with only *Giardia lamblia* infection were isolated, the sample size was reduced to 18 patients, of whom, only 8 were randomized to NTZ (just one patient out of 8 was female). These sample sizes were very small. Also, kappa coefficient for the NTZ arm in the group of patients with giardiasis was negative (-0.283), compromising the meaningfulness of the parasitological endpoint.

The other study was active (metronidazole) controlled study in children 2 to 11 years of age for NTZ suspension in Peru. In this study, the non-inferiority of NTZ compared to metronidazole with a margin of 20% was demonstrated with 95% confidence in terms of the clinical response rates (85% for NTZ versus 80% for Metronidazole), but not in terms of the parasitological response rates. The kappa coefficients for both NTZ and metronidazole arms were positive and comparable (0.276 for NTZ, 0.227 for Metronidazole). All subgroup/special protocol analyses in this study, such as ones based on gender, subgroup of patients with only *Giardia lamblia* infection, "per protocol" population, all showed results consistent with the Intent-to-Treat population.

The study reviewed in this document was the third study of the efficacy of tablets for the treatment of diarrhea caused by *Giardia lamblia*. This study was randomized, double-blind, placebo-controlled study conducted in one center in Egypt and one center in Peru in adults and adolescents (age > 11 years). This study showed that 7-10 day clinical response rate for NTZ tablets (85%) was statistically significantly better than placebo (44%) (p-value = 0.0002). The 95% confidence interval for the difference in clinical response rates for the tablet and suspension was (-14%, 17%). Parasitological response rate at day 7-10 visit for NTZ tablets, although statistically significantly different from placebo, correlated weakly to the clinical response rate as measured by kappa coefficient (kappa = 0.196) and the difference in cyst counts in concentrated stool samples from baseline to day 7-10 was not statistically significantly different from placebo.

Thus, collectively these studies demonstrate that NTZ is effective in suspension


formulation in children and in both tablet and suspension formulation in adults and adolescents for the treatment of diarrhea caused by *Giardia lamblia* in terms of only the clinical response. The evidence presented in these studies is not adequate for efficacy in terms of parasitological response

5.2 Conclusions and Recommendations

The efficacy of NTZ tablets for the treatment of diarrhea caused by *Giardia lamblia* in adults and adolescents (≥ 12 years of age) was studied in this application, in a randomized, double-blind, placebo-controlled study conducted in one center in Egypt and one center in Peru.

This study demonstrated superior efficacy of NTZ tablets over placebo in terms of 7-10 day clinical response rate (defined as 'no symptoms, no watery stools and no more than 2 soft stools with no hematochezia within the past 24 hours' or 'no symptoms and no unformed stools within the past 48 hours'). The 95% confidence interval for the difference in clinical response rates for the tablet and suspension was (-14%, 17%).

Parasitological response rate at day 7-10 visit for NTZ tablets, although statistically significantly better than placebo, correlated weakly to the clinical response rate as measured by kappa coefficient ($\kappa = 0.196$) and the difference in cyst counts in concentrated stool samples from baseline to day 7-10 was not statistically significantly different from placebo. Parasitological response at day 14-17, again although statistically significantly better than placebo in clinical responders at day 7-10 was not accompanied by clinical evaluation and showed some worsening in terms of cyst counts in concentrated stool samples for NTZ tablets, especially at the center in Peru. These results are inconclusive due to small sample sizes.

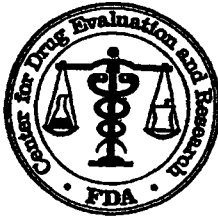


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DEPARTMENT OF HEALTH AND HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH
OFFICE OF BIostatISTICS

Statistical Review and Evaluation

CLINICAL STUDIES

NDA: 21-497 / 21-498

Name of drug: nitazoxanide 500 mg tablets and nitazoxanide 100mg / 5ML oral suspension.

Drug Regimen: b.i.d. Treatment Duration: 3 days

Applicant: Romark Laboratories, L.C.

Indication: Treatment of Diarrhea caused by *Cryptosporidium parvum*,
Giardia lamblia

Clinical Division: Division of Special Pathogen and Immunologic Drug Products (DSPIDP), HFD-590

Project manager: Kristen Miller (DSPIDP, HFD-590)

Clinical reviewer: Rosemary Johann-Liang, M.D. (DSPIDP, HFD-590)

Dates: Submission 5/29/02; 45 day meeting 6/21/02; user fee (6 months) 11/29/02.

Documents reviewed: Volumes 1.1 and 1.32-1.34, 1.38-1.39 and electronic submission found under
\\CDSesub1\N21497\N_000\2001-05-29

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Keywords: Clinical Studies, NDA review, Single-Center Studies, Superiority Trials, Non-inferiority Trials, Kappa coefficient

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1 EXECUTIVE SUMMARY OF STATISTICAL FINDINGS

1.1 CONCLUSIONS AND RECOMMENDATIONS

The submissions, NDA 21-497 and NDA 21-498, by Romark Laboratories include five adequate and well-controlled clinical studies that were designed and conducted using the proposed three-day treatment regimen to demonstrate the efficacy of nitazoxanide (NTZ) in treating diarrhea caused by *C. parvum* and *G. lamblia*. All five studies were conducted at single centers in endemic areas, namely Egypt, Zambia and Peru. Four of these five studies were randomized, double-blind, placebo-controlled studies and one study was randomized, single-blind active-controlled study with metronidazole as comparator. The studies involved adults, children and in the case of diarrhea caused by *C. parvum*, children with malnutrition and children with AIDS. Both clinical and parasitological endpoints were used to evaluate efficacy. The responses were measured approximately 4 days after the end of treatment. For the clinical endpoint, 'well' response was defined as 'no symptoms, no watery stools and no more than 2 soft stools with no hematochezia within the past 24 hours' or 'no symptoms and no unformed stools within the past 48 hours.' For the parasitological endpoint, 'Eradication' was defined as 'no cysts of the parasite observed in either of the 2 post-treatment parasitological examinations'. Efficacy data was analyzed using intent-to treat (ITT) population generally defined as all patients who took at least one dose of the treatment.

Single Center Studies

All five studies in this application were designed as single center, single race studies conducted outside of Unites States. This fact seriously compromises the generalizability of the results of these studies to the population in Unites States, and applicability of these results to future non-inferiority studies where NTZ, if approved, may be used as an active comparator. The data from these studies could not be used to assess the effects of important covariates such as race, age group, pathogen, and the interactions of these covariates among each other as these effects were confounded with the study designs and study to study variability.

It is strongly recommended that any future studies in the development of NTZ be designed and conducted as adequate, well-controlled, multi-center studies.

C. Parvum

The data in HIV negative adult population did not provide adequate evidence of efficacy of NTZ tablets for the treatment of Diarrhea caused by *Cryptosporidium parvum*.

However, the observed trends in the clinical and parasitological response rates were in favor of NTZ.

It is recommended that any future studies be conducted as placebo controlled, multi-center studies in patients with only *C. Parvum* infection. An evaluation and interpretation of correlation between clinical and parasitological responses and its implications should be addressed in the data analyses.

The data in the population of HIV negative children provided adequate evidence of efficacy of NTZ suspension for the treatment of Diarrhea caused by *C. parvum*, —

Giardia Lamblia

The data in HIV negative adult population did not provide adequate evidence of efficacy of NTZ tablets for the treatment of Diarrhea caused by *Giardia Lamblia* —

It is recommended that any future studies be conducted as placebo controlled, multi-center studies in patients with only *Giardia Lamblia* infection. An evaluation and interpretation of correlation between clinical and parasitological responses and its implications should be addressed in the data analyses.

The data in children's population provided adequate evidence of efficacy of NTZ suspension for the treatment of Diarrhea caused by *Giardia Lamblia*, —

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1.2 OVERVIEW OF CLINICAL PROGRAM AND STUDIES REVIEWED

Nitazoxanide was originally synthesized in the early 1970s, but was not fully developed at that time. Romark Laboratories re-initiated the development of nitazoxanide in the 1990s for treating infections caused by a broad spectrum of parasites that infect intestinal tracts of humans including *Cryptosporidium parvum* infections. The current submissions, NDA 21-497 and NDA 21-498, include five adequate and well-controlled clinical studies that were designed and conducted using the proposed three-day treatment regimen to demonstrate the efficacy of nitazoxanide in treating diarrhea caused by *C. parvum* and *G. lamblia*. Both of these diseases are considered orphan diseases in the United States.

All five studies were conducted at single centers in endemic areas, namely Egypt, Zambia and Peru. Four of these five studies were randomized, double-blind, placebo-controlled studies and one study was randomized, single-blind active-controlled study with metronidazole as comparator.

The studies involved adults, children and in the case of diarrhea caused by *C. parvum*, children with malnutrition and children with AIDS.

All studies were designed similarly and used both clinical and parasitological endpoints to evaluate efficacy. Efficacy data was analyzed using an intent-to-treat (ITT) population generally defined as all patients who took at least one dose of the treatment.

The following table provides a brief overview of the five pivotal studies.

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Table 1: Controlled Clinical Trials in NDAs 21-497 and 21-498

| Study # | Center | Study Design | Treatment: dose | Sample size | Age range | Treatment Duration |
|--|--------|--------------------|-----------------|-------------|-----------|--------------------|
| Controlled Clinical Trials : Diarrhea Caused by C. Parvum | | | | | | |
| RM-NTZ 98-002 | Egypt | Randomized | NTZ: | | | 3 days |
| | | double-blind | 100 mg bid | 11 | 1-3 | |
| | | Placebo-controlled | 200 mg bid | 13 | 4-11 | |
| | | | 500 mg bid | 25 | 12-62 | |
| | | Placebo:bid | 50 | 1-62 | 3 days | |
| RM02-3007 | Zambia | Randomized | NTZ: | | | 3 days |
| | | double-blind | 100 mg bid | 25 | 1-3 | |
| | | Placebo-controlled | Placebo:bid | 22 | 1-3 | 3 days |
| RM02-3008 | Zambia | Randomized | NTZ: | | | 3 days |
| | | double-blind | 100 mg bid | 24 | 1-3 | |
| | | Placebo-controlled | 200 mg bid | 1 | 4-11 | |
| | | | Placebo:bid | 25 | 1-3 | 3 days |
| Controlled Clinical Trials : Diarrhea Caused by Giardia lamblia | | | | | | |
| RM-NTZ 98-001 | Egypt | Randomized | NTZ: | | | 3 days |
| | | double-blind | 500 mg bid | 47 | 12-65 | |
| | | Placebo-controlled | Placebo:bid | 44 | 12-65 | 3 days |
| RM-NTZ 99-010 | Peru | Randomized | NTZ: | | | 3 days |
| | | single-blind | 100 mg bid | 14 | 2-3 | |
| | | Active-controlled | 200 mg bid | 41 | 4-11 | |
| | | | Metronidazole | | | 5 days |
| | | 125 mg bid | 29 | 2-3 | | |
| | | 250 mg bid | 26 | 4-11 | | |

1.3 PRINCIPLE FINDINGS

C. Parvum

Three studies were conducted by the sponsor to demonstrate the efficacy of three-day regimen of NTZ in treating diarrhea caused by *C. Parvum*.

Adult Study Results

Adult HIV negative population (age ≥ 12 years) was studied in only one study, RM-NTZ-98-002, conducted at a single center in Egypt.

Single center, single race nature of this study seriously compromises the generalizability of the results of this study to the general population.

The difference between NTZ and placebo for the clinical endpoint ('well' response defined as 'no symptoms, no watery stools and no more than 2 soft stools with no hematochezia within the past 24 hours' or 'no symptoms and no unformed stools within the past 48 hours.') was not statistically significant (p-value=0.0423, one-sided). A statistically significant finding was reported for the parasitological endpoint ('Eradication' defined as 'no cysts of the parasite observed in either of the 2 post-treatment parasitological examinations'). However, poor correlation between clinical and parasitological endpoints for the NTZ arm (kappa = -0.312) seriously compromises the meaningfulness of parasitological endpoint for this population.

Moreover, there were 8 patients (4- placebo, 4-NTZ) with mixed infections. When the analyses were carried out by excluding these patients (that is, for the adult population with only *C. Parvum* infection), the differences between NTZ and placebo were not statistically significant for both clinical and parasitological endpoints (p-values =.118). Subgroup analyses by gender, generally, did not show statistically significant difference between NTZ and Placebo for both clinical and parasitological endpoints.

Therefore, the data in HIV negative adult population did not provide adequate evidence of efficacy of NTZ tablets for the treatment of Diarrhea caused by *Cryptosporidium parvum*. However, the observed trends in the clinical and parasitological response rates were in favor of NTZ.

It is recommended that any future studies be conducted as placebo controlled, multi-center and in patients with only *C. Parvum* infection. An evaluation and interpretation of correlation between clinical and parasitological responses and its implications should be addressed in the data analyses.

Pediatric Studies Results

The population of HIV negative children (age 1-11 years) was studied in two randomized placebo controlled studies, one, RM-NTZ-98-002, conducted at a single center in Egypt and another, RM02-3007, conducted at a single center in Zambia.

Single center, single race nature of these studies seriously compromises the generalizability of the results of these studies to the general population. The data from these studies could not be used to assess the effects of important covariates such as race, malnourishment status, pathogen, and the interactions of these covariates among each other as these effects were confounded with the study designs and study to study variability.

There were subtle differences in these two studies as follows. Ages of children in study RM-NTZ-98-002 ranged from 1-11 years, whereas the ages of children in study RM02-3007 ranged from 1 to less than 4 years, and all but two children in study RM02-3007 were malnourished. In study RM-NTZ-98-002, presence of *C. Parvum* at baseline was not re-confirmed, whereas in study RM02-3007, it was re-confirmed at baseline, and 3 male patients randomized to placebo group with no oocysts of *C. Parvum* at baseline were excluded from all efficacy analyses by the sponsor.

In both studies, the differences between NTZ and placebo were statistically significant (in favor of NTZ) in terms of clinical response (88% versus 38% in Study RM-NTZ-98-002 and 56% versus 23% in Study RM02-3007) and parasitological response (75% versus 20% in Study RM-NTZ-98-002 and 52% versus 14% in Study RM02-3007). However, sensitivity analyses and subgroup (based on age and gender)/special (only *C. Parvum* infection) population analyses did not, consistently, show statistically significant results. In both studies, kappa coefficients were positive for NTZ arm, but were not consistent across studies, age subgroups, gender subgroups and for the population of patients with only *C. Parvum* infection.

In study RM02-3007, there was statistically significant difference in the mortality rate within 7 days following enrollment between NTZ and Placebo (0% versus 18%, respectively).

The data in the population of HIV negative children provided adequate evidence of efficacy of NTZ suspension for the treatment of Diarrhea caused by *C. parvum*.

It is recommended that any future studies be conducted as placebo controlled, multi-center and in patients with only *C. Parvum* infection. An evaluation and interpretation of correlation between clinical and parasitological responses and its implications should be addressed in the data analyses.

Another double-blind, placebo-controlled study was conducted in hospitalized, severely malnourished children of age 1-11 years with acquired immune deficiency syndrome (AIDS) in Zambia. In this study, a three-day course of nitazoxanide suspension (100 mg BID in children ages 12-47 months, 200 mg BID in children ages 4 through 11 years) did not produce clinical or parasitological response rates or mortality rates that were significantly different from the placebo control. Aside from their infection with HIV, the patients enrolled in this study were significantly more malnourished (mean weight-for-age z-scores: -5.5 compared to -3.5, $p < 0.0001$); they reported a longer duration of diarrhea at time of enrollment (49 days compared to 20 days, $p < 0.01$); and they had lower CD4 cell counts

(mean: 620 cells/mm³ compared to 1532 cells/mm³, p<.0001) than those of HIV negative children enrolled in study RM02-3007.

*Giardia Lambli*a

Adult Study Results

Adult HIV negative population with *Giardia Lambli*a was studied in only one placebo controlled study, RM-NTZ-98-001, conducted at a single center in Egypt.

Single center, single race nature of this study seriously compromises the generalizability of the results of this study to the general population.

Moreover, this study enrolled patients with either giardiasis or amoebiasis or both. Also 17 patients (8-NTZ, 9-placebo) had other additional infections. In this study, when patients infected with only *Giardia Lambli*a infection were isolated, the sample size was reduced to 18 patients, of whom, only 8 were randomized to NTZ (just one patient out of 8 was female). These sample sizes were very small. Therefore the results of this single center, single race study, although statistically significant, could not be generalized. Also, kappa coefficient for the NTZ arm in the group of patients with giardiasis was negative (-0.283), compromising the meaningfulness of the parasitological endpoint (as in case of the study RM-NTZ-98-002 in *C. Parvum* infection).

The data in HIV negative adult population did not provide adequate evidence of efficacy of NTZ tablets for the treatment of Diarrhea caused by *Giardia Lambli*a

It is recommended that any future studies be conducted as placebo controlled, multi-center and in patients with only *Giardia Lambli*a infection. An evaluation and interpretation of correlation between clinical and parasitological responses and its implications should be addressed in the data analyses.

Pediatric Study Results

The population of HIV negative children (age 2-11years) with *Giardia Lambli*a infection was studied in only one active controlled study, RM-NTZ-99-010, conducted at a single center in Peru, where metronidazole was used as an active control.

Single center, single race nature of this study seriously compromises the generalizability of the results of this study to the general population.

In this study, the non-inferiority of NTZ compared to metronidazole with a margin of 20% was demonstrated with 95% confidence in terms of the clinical response rates (85% for NTZ versus 80% for Metronidazole) , but not in terms of the parasitological response rates. The kappa coefficients for both NTZ and metronidazole arms were positive and comparable (0.276 for NTZ, 0.227 for Metronidazole). All subgroup/special protocol analyses in this study, such as ones based on gender, subgroup of patients with only *Giardia Lambli*a

infection, "per protocol" population, all showed results consistent with the Intent-to Treat population.

The data in children's population provided adequate evidence of efficacy of NTZ suspension for the treatment of Diarrhea caused by *Giardia Lambli*a,

It is recommended that any future studies be conducted as placebo controlled, multi-center and in patients with only *Giardia Lambli*a infection. An evaluation and interpretation of correlation between clinical and parasitological responses and its implications should be addressed in the data analyses.

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2 INTRODUCTION

2.1 BACKGROUND

Nitazoxanide was originally synthesized in the early 1970s, but was not fully developed at that time. Romark Laboratories re-initiated the development of nitazoxanide (NTZ) in the 1990s for treating infections caused by a broad spectrum of parasites that infect intestinal tracts of humans including *Cryptosporidium parvum* infections.

In 1994, a program was initiated in 7 different sites in Egypt to evaluate efficacy and safety of NTZ. The focus was on patient's parasitological response to the treatment. The first dose regimen tested was 500 mg bid for three days in adults. After the safety of the drug had been established in approximately 200 adults, children were added to the study with the dose adjusted to 200 mg for age 4-11 years and to 100 mg for age 1-3 years. The 3-day regimen produced high eradication rates for a broad spectrum of protozoa and helminths.

To confirm the efficacy of the 3-day course of NTZ in treating intestinal parasitic infections, another uncontrolled study was conducted in adults and children in Mexico with mixed infections by protozoa and helminths (at least 3 parasites per subject). Again the focus was patients' parasitological response to treatment. The findings of this study were consistent with those of the Egyptian study.

The current submissions, NDA 21-497 and NDA 21-498, include five adequate and well-controlled clinical studies that were designed and conducted using the proposed three-day treatment regimen to demonstrate the efficacy of nitazoxanide in treating diarrhea caused by *C. parvum* and *G. lamblia*. Both of these diseases are considered orphan diseases in the United States. Currently there are no approved drug products labeled for the treatment of diarrhea caused by *C. Parvum*.

All of the five studies were designed based on guidelines published in the literature(1) for the evaluation of new drugs for treating diarrhea caused by *Giardia lamblia*. All patients selected for inclusion in these studies were symptomatic with diarrhea and were diagnosed with the enteric protozoan pathogen under study by stool examination. The studies involved adults, children and in the case of diarrhea caused by *C. parvum*, children with malnutrition and children with AIDS.

Four of these five studies were randomized, double-blind, placebo-controlled studies and one study was randomized, single-blind active-controlled study with metronidazole as comparator.

Both clinical and parasitological endpoints were used to evaluate efficacy. The responses were measured approximately 4 days after the end of treatment. For the clinical endpoint, 'well' response was defined as 'no symptoms, no watery stools and no more than 2 soft stools with no hematochezia within the past 24 hours' or 'no symptoms and no unformed

stools within the past 48 hours.' For the parasitological endpoint, 'Eradication' was defined as 'no cysts of the parasite observed in either of the 2 post-treatment parasitological examinations'.

Efficacy data was analyzed using intent-to treat (ITT) population in all the studies. However, in studies RM-NTZ-98-001 and RM-NTZ-98-002, the protocols allowed the diagnosis within 7 days prior to enrollment and all patients enrolled had diarrhea at the time of enrollment, but the parasitological diagnosis was not reconfirmed at baseline. In the other three studies, the diagnosis was re-confirmed at baseline, and patients for whom the diagnosis was not reconfirmed were excluded from the ITT population and therefore from the efficacy analyses.

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2.2 DATA ANALYZED AND SOURCES

In order to recruit a sufficient number of patients to evaluate the efficacy of NTZ, all five pivotal studies were conducted in endemic areas, namely, Egypt, Peru and Zambia. All five studies were single center studies. The sponsor stated that the following efforts were made to ensure the quality of the studies:

- 1> The sites selected were willing to work with a team of foreign investigators and had patient availability, personnel and facilities that would allow completion of a study over a 12-month period.
- 2> Staff on site included a physician qualified in tropical medicine and one clinical research associate.
- 3> Additional personnel including physicians, parasitologists and clinical research associates were sent to the sites for short time periods to conduct study monitoring and quality assurance of parasitological examinations.
- 4> Romark's Quality Assurance staff audited the studies to assure compliance with the protocols.

The data sets for all five studies were submitted electronically at the following location:

\\CDSESUB1\N21497\N_000\2001-05-29\CRT\Datasets

This reviewer found the efficacy data sets to be well organized and of good quality. Also this reviewer did not find notable discrepancies between the results given in the text of the sponsor's study report and those obtained using the submitted data sets.

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3 STATISTICAL EVALUATION OF EVIDENCE ON EFFICACY / SAFETY

3.1 DIARRHEA CAUSED BY *C. PARVUM*

3.1.1 STUDY DESIGN

The sponsor conducted three randomized, double-blind, placebo-controlled studies to evaluate efficacy of NTZ in the treatment of diarrhea caused by *C. Parvum*. Dosing and study design information is given in Table 1. Study RM-NTZ-98-002 was conducted in adults and children at a single center in Egypt, study RM02-3007 was conducted in HIV negative children (all but two malnourished) at a single center in Zambia, and study RM02-3008 was conducted in HIV positive, severely malnourished, hospitalized children at a single center in Zambia. All patients enrolled had diarrhea at the time of enrollment (criteria given in Table 2) and oocysts of *C. parvum* had been identified in a stool sample collected within 7 days prior to enrollment. Parasitological diagnosis was reconfirmed at baseline in studies RM02-3007 and RM02-3008 (but not in Study RM-NTZ-98-002). Patients in these two studies for whom the diagnosis was not reconfirmed were excluded from the efficacy analyses.

Upon enrollment, the patients were randomized to either NTZ or Placebo arms, and received 3 consecutive days of treatment. In study RM-NTZ-98-002, randomization was stratified by age group (1-11 years, >11 years). A post-treatment physical exam was performed on day 7 (\pm 2 days). Two post-treatment fecal samples were obtained from each patient between day 7 and 10 (\pm 2 days). Each fecal sample was subjected to a parasitological exam. The criteria for evaluating clinical (Well, Continuing Illness, Clinical Failure) and parasitological (Eradication, Persistence) responses were clearly stated in the protocol. Patients with bacterial causes of diarrhea were excluded from the analysis of clinical response. However, patients with other intestinal parasitic infections were not excluded from the study, due to high prevalence of intestinal parasitic infections in the Nile Delta Region and the potential difficulties in enrolling patients infected by *C. Parvum* alone.

The sample size for each of these studies was calculated (with an estimated 20% drop out rate) to provide 80% power to detect a difference between an 85% response rate for the active treatment group and a 40% response rate for the placebo group using a one sided alpha of 0.025. Study RM-NTZ-98-002 was powered to detect a difference in adults as well as children.

3.1.2 EFFICACY ENDPOINTS

All three studies evaluated two efficacy endpoints: **clinical response** (well, continuing illness, clinical failure) on study day 7 (4 days after the end of treatment) and **parasitological response** (eradication or persistence) based on two stool samples collected between study days 7 and 10. For the clinical endpoint, 'well' response was defined as 'no symptoms, no watery stools and no more than 2 soft stools with no hematochezia within the past 24 hours'

or 'no symptoms and no unformed stools within the past 48 hours.' For the parasitological endpoint, 'Eradication' was defined as 'no cysts of the parasite observed in either of the 2 post-treatment parasitological examinations'.

In Study RM-NTZ-98-002, both of these endpoints were stated as primary endpoints, where as in Studies RM02-3007 and RM02-3008, clinical response was stated as primary endpoint and parasitological response was stated as secondary endpoint. Time from initiation of treatment to passage of last unformed stool (as reported by the patient in a diary) was evaluated as secondary efficacy endpoint in all three studies.

Reviewer's Comments

- 1> *Since the sponsor is seeking indications of "Treatment of Diarrhea" both clinical response and parasitological response are treated as primary endpoints in this review.*
- 2> *It was stated in the protocol for study RM-NTZ-98-002 that _____ is would be analyzed as a secondary endpoint. However, this was not done in the final study report. No explanation for this change was given. The data sets submitted with the application did not contain any information about this endpoint. Hence this review does not contain any discussion of the results concerning this endpoint. This endpoint was not considered in any of the other protocols in this NDA*

3.1.3 STATISTICAL METHODOLOGY USED BY THE SPONSOR

Fisher's exact tests were used to compare the treatment groups in terms of clinical and parasitological response rates. The median time from initiation of therapy to date of last unformed stool was compared for the active and placebo treatment groups using Kaplan-Meier survival analysis. The sponsor also used the Median test for the observed data to test the null hypothesis that the two treatment groups have the same median time to last unformed stool.

Reviewer's Comment

Median test is a chi-squared test applied to the 2×2 contingency table constructed in such a way that the two entries in the first column are the number of observations for treatment 1 (say placebo) that are above and below the grand median (the median of all observations the both treatment groups combined), and the two entries in the second column are the number of observations for treatment 2 (say NTZ) that are above and below the grand median. For more discussion of this test the reader is referred to the reference (4).

3.1.4 DIFFERENCES/SIMILARITIES IN THE THREE STUDIES

The following table shows differences/similarities among the three studies in terms of location, study design, patient population and other characteristics.

Table 2: Controlled Clinical Trials for *C. Parvum*

| | RM-NTZ-98-002 | RM02-3007 | RM02-3008 |
|--|--|--|--|
| Center | Egypt | Zambia | Zambia |
| Patient population | Adults (age \geq 12 years) and Children (age < 12 years) | Children (age < 3 years), all but two malnourished | Children (age < 12 years), all malnourished |
| HIV status | Negative | Negative | Positive |
| Diarrhea | > 4 stools /day | \geq 3 stools per day | \geq 3 stools per day |
| Parasitological diagnosis | Not confirmed at Baseline (enrollment) | Confirmed at Baseline(enrollment) | Confirmed at Baseline(enrollment) |
| Excluded from efficacy analyses | Patients with Bacterial infections | 1> Patients with bacterial infections 2> Patients with no oocysts at baseline | 1> Patients with bacterial infections 2> Patients with no oocysts at baseline |

3.1.5 PATIENT DISPOSITION – ALL THREE STUDIES

Table 3: Patient disposition for Controlled clinical trials for *C. Parvum*

| | RM-NTZ-98-002 | | RM02-3007 | | RM02-3008 | |
|---|----------------------|----------------|------------------|----------------|------------------|----------------|
| SCREENED | 725 | | Not Reported | | Not Reported | |
| | NTZ | Placebo | NTZ | Placebo | NTZ | Placebo |
| ENROLLED | 50 | 50 | 25 | 25 | 25 | 25 |
| COMPLETED | 49 | 50 | 25 | 19 | 20 | 21 |
| WITHDRAWN | | | | | | |
| Withdrew Consent | 1 | 0 | None | | None | |
| No baseline parasite | Not Tested | | 0 | 3* | None | |
| AE | None | | 0 | 1 | None | |
| Death | None | | 0 | 4 | 5 | 4 |
| OTHER INFECTIONS | | | | | | |
| Bacterial | 0 | 1 | None | | 0 | 1 |
| Parasitic | 13 | 7 | 1 | 0 | None | |
| PROTOCOL DEVIATIONS | | | | | | |
| <i>C. Parvum</i> diagnosis more than 7 days prior to enrollment | 9 | 11 | None | | None | |
| Late or No show for Post Rx exam Day 7 | 4 | 4 | None | | None | |
| Late or No show for Post Rx exam Day 10 | 6 | 12 | 1 | 1 | None | |
| * one patient was withdrawn by investigator and 2 remained in the study | | | | | | |

3.1.6 DEMOGRAPHICS AND BASELINE CHARACTERISTICS – ALL THREE STUDIES

The sponsor summarized demographic and disease related characteristics for all the subjects in each study regardless of their treatment assignment and noted that statistically significantly higher number of children reported liquid stools compared to adults.

Reviewer's Comment:

This reviewer summarized demographic and disease related characteristics in the following tables by the treatment group. These were generally comparable across the treatment groups.

Table 4A: Demographics and Baseline Characteristics - Adults

| ADULTS | | | | | | |
|---|----------------------|------------------|--|----------------|------------------|----------------|
| Demographic or Disease Related Characteristics | STUDY | | | | | |
| | RM-NTZ-98-002 | | RM02-3007 | | RM02-3008 | |
| | NTZ | Placebo | NTZ | Placebo | NTZ | Placebo |
| Male | 12 | 15 | ADULTS WERE NOT STUDIED IN THESE TRIALS | | | |
| Female | 13 | 10 | | | | |
| Age (yrs)– Mean (s.d.) | 35.52 (12.44) | 34.92 (12.62) | | | | |
| Weight (kgs)– Mean (s.d.) | 70.60 (15.24) | 73.56 (14.37) | | | | |
| Stool Frequency | | | | | | |
| 5-10 stools /day | 25 | 25 | | | | |
| > 10 stools /day | 0 | 0 | | | | |
| Stool Consistency | | | | | | |
| Liquid | 9 | 2 | | | | |
| Semisolid | 16 | 23 | | | | |
| Duration of Diarrhea- Median no. of days | 9 | 10 | | | | |

Table 4B: Demographics and Baseline Characteristics - Children

| CHILDREN | | | | | | |
|---|----------------------|-----------------|------------------|----------------|--------------------|--------------------|
| Demographic or Disease Related Characteristics | STUDY | | | | | |
| | RM-NTZ-98-002 | | RM02-3007 | | RM02-3008 | |
| | NTZ | Placebo | NTZ | Placebo | NTZ | Placebo |
| Male | 13 | 16 | 16 | 18 | 12 | 15 |
| Female | 12 | 9 | 9 | 7 | 13 | 10 |
| Age (yrs)- Mean (s.d.) | 5.34 (3.27) | 5.0 (3.20) | 1.24 (.44) | 1.2 (.41) | 21.76 (7.03) | 27.04 (15.68) |
| Weight (kgs)- Mean (s.d.) | 20.25 (7.95) | 18.36 (8.17) | 8.37 (1.53) | 8.28 (1.28) | 7.13 (1.49) | 7.96 (2.7) |
| Stool Frequency | | | | | | |
| 3-4 stools/day | 0 | 0 | 2 | 3 | 0 | 0 |
| 5-10 stools /day | 23 | 23 | 23 | 21 | 24 | 25 |
| > 10 stools /day | 2 | 2 | 0 | 1 | 1 | 0 |
| Stool Consistency | | | | | | |
| Liquid | 10 | 18 | 17 | 20 | 15 | 20 |
| Semisolid | 15 | 7 | 4 | 2 | 4 | 2 |
| Liquid & Semisolid | 0 | 0 | 4 | 3 | 5 | 3 |
| Duration of Diarrhea- Median no. of days | 9 | 9 | 18 | 10.5 | 29 | 21 |
| CD4 count - Mean (s.d.) | N/A | N/A | N/A | N/A | 618.59 (445.55) | 620.81 (631.66) |

3.1.7 SPONSOR'S EFFICACY RESULTS

3.1.7.1 Study RM-NTZ-98-002

Out of 100 patients enrolled in the study, the parent of one child randomized to NTZ decided not to participate in the study and returned all of the study medication on the day of enrollment. This patient was excluded from all efficacy analyses conducted by the sponsor. Also the sponsor excluded one patient (patient number 33C) randomized to placebo with a positive coproculture for bacterial cause of diarrhea from the analysis of clinical response, in accordance with the protocol. The following table shows sponsor's primary analyses for the clinical and parasitological response using Fisher's exact tests for the Intent-To-Treat (ITT) population. The sponsor stated that precise median time to last unformed stool for the placebo group could not be determined since patients were not followed up past the date of post-treatment examination (day 6).

Table 5. Sponsor’s Efficacy results for Study RM-NTZ-98-002 – ITT population

| Study Medication | “Well” Clinical Response | “Eradicated” Parasitological Response | Median time to last unformed stool |
|---------------------|--------------------------|---------------------------------------|------------------------------------|
| ADULTS | | | |
| NTZ | 18/25 (72%) | 15/25 (60%) | 3 days |
| Placebo | 11/25 (44%) | 6/25 (24%) | 6 days |
| p-value (one sided) | .0423 | .0104 | .0493 |
| CHILDREN | | | |
| NTZ | 21/24 (88%) | 18/24 (75%) | 3.5 days |
| Placebo | 9/24 (38%) | 5/25 (20%) | 6 days |
| p-value (one sided) | .0004 | .0001 | .0001 |

Reviewer’s Comments:

- 1> *The application consists of two NDA’s: 21-497 for NTZ tablets for adults and 21-498 for NTZ pediatric suspension for children. This reviewer will treat “Adults” and “Children” as two different patient populations and will review the data separately. This is consistent with the study design as the study was powered for adults and children separately.*
- 2> *The p-values in the above Table 5 are one-sided and statistical significance is concluded if p-value < 0.025.*
- 3> *For Adults (ITT population), the data did not provide statistically significant evidence of a difference between placebo and NTZ in terms of clinical response and median time to last unformed stool (p-values are > 0.025).*
- 4> *The sponsor also conducted “per protocol” analyses and results were consistent with those in Table 5.*
- 5> *This reviewer conducted several analyses to evaluate the evidence based on age group in children, gender, and patients with only C. Parvum infection. (See Section 3.3). Also this reviewer investigated the correlation between clinical and parasitological response extensively (See Sections 3.1.8 and 3.3).*

3.1.7.2 Study RM02-3007

Out of 50 patients enrolled in the study, 3 patients (all in placebo group, all male) with no oocysts of *C. Parvum* in the stool sample collected at baseline were excluded by the sponsor from all efficacy analyses. The following table shows sponsor’s primary analyses for the clinical and parasitological response using Fisher’s exact tests for the Intent-To-Treat (ITT) population (consisting of 47 patients). The time to last unformed stool was compared using Kaplan-Meier curves and log-rank test.

Table 6. Sponsor’s Efficacy results for Study RM02-3007 – ITT population

| Study Medication | “Well” Clinical Response | “Eradicated” Parasitological Response | Time to last unformed stool |
|---------------------|--------------------------|---------------------------------------|---|
| CHILDREN | | | |
| NTZ | 14/25 (56%) | 13/25 (52%) | Can’t be determined - More than 50% censoring |
| Placebo | 5/22 (23%) | 3/22 (14%) | Can’t be determined - More than 50% censoring |
| p-value (two sided) | .0362 | .0069 | .278 |

Reviewer’s Comments:

- 1> In this study, there was statistically significant difference in the mortality rate within 7 days following enrollment between NTZ and Placebo (0% versus 18%, respectively).***
- 2> The p-values in the above Table 6 are two sided and statistical significance is concluded if p-value < 0.05.***
- 3> The sponsor’s analysis of time to last unformed stool has an error. This reviewer re-analyzed the data and the correct p-value is 0.46. This correction does not change the conclusion of no statistically significant difference between NTZ and placebo groups in terms of Time to Last Unformed Stool.***
- 4> This reviewer conducted several analyses to evaluate the evidence based on gender, and patients with only C. Parvum infection. (See Section 3.3). Also this reviewer investigated the correlation between clinical and parasitological response extensively (See Sections 3.1.8 and 3.3).***
- 5> Out of 50 patients enrolled in this study, 3 patients (all in placebo group, all male) with no oocysts of C. Parvum in the stool sample collected at baseline were excluded by the sponsor from all efficacy analyses. Since these patients were treated and the clinical response and parasitological response was observed for all three patients, this reviewer conducted sensitivity analyses by including these patients. In these analyses, statistical significance was not reached for both endpoints (see section 3.3.2 for details and further discussion).***

3.1.7.3 Study RM02-3008

Out of 50 patients enrolled in the study, 1 patient (placebo group) with bacterial cause of diarrhea (Salmonella) was excluded by the sponsor from efficacy analyses of clinical response. The following table shows sponsor’s primary analyses for the clinical and

parasitological response using Fisher's exact tests for the Intent-To-Treat (ITT) population. The time to last unformed stool was compared using Kaplan-Meier curves and log-rank test.

Table 7. Sponsor's Efficacy results for Study RM02-3008 – ITT population

| Study Medication | "Well" Clinical Response | "Eradicated" Parasitological Response | Time to last unformed stool |
|---------------------|--------------------------|---------------------------------------|---|
| CHILDREN | | | |
| NTZ | 2/25 (8%) | 4/25 (16%) | Can't be determined - More than 50% censoring |
| Placebo | 6/24 (25%) | 5/25 (20%) | Can't be determined - More than 50% censoring |
| p-value (two sided) | .1383 | 1.0 | .07 |

Reviewer's Comment:

- 1> *Statistically significant benefit of NTZ was not demonstrated in this study. No relationship between demographic or disease related characteristics and the efficacy endpoints was observed.*
- 2> *The sponsor pointed out that aside from their infection with HIV, the patients enrolled in this study were significantly more malnourished (mean weight-for-age z-scores: -5.5 compared to -3.5, $p < 0.0001$); they reported a longer duration of diarrhea at time of enrollment (49 days compared to 20 days, $p < 0.01$); and they had lower CD4 cell counts (mean: 620 cells/mm³ compared to 1532 cells/mm³, $p < .0001$) than HIV negative children enrolled in study RM02-3007.*

3.1.8 CORRELATION BETWEEN CLINICAL RESPONSE AND PARASITOLOGICAL RESPONSE

Multiple endpoints are often used in clinical trials to characterize drug benefit in several ways or to characterize different aspects of drug benefit. These endpoints may be correlated weakly or strongly. Kappa Coefficient is a quantitative measure of reproducibility of drug benefit measured with two nominal endpoints. Theoretical Range of Kappa is [-1, 1] with higher positive values indicating higher concordance. The use of kappa to evaluate the correlation between clinical and parasitological response is discussed in Section 3.4.

In this application, for the NTZ arm, positive kappa values are expected in order to consider parasitological response to be a meaningful endpoint. For the placebo arm, near zero kappa values are expected since spontaneous resolution of symptoms is observed in patients with *C. Parvum* and the possibilities of diarrhea from infections other than *C. Parvum* infection exist.

Kappa Coefficients for all the three studies are provided in the following table.

Table 8: Kappa Coefficients

| Study | Population | Treatment | N | Kappa | No. of discordant pairs (well & persistence + ill & eradicated) |
|------------------|------------------------|-----------|-----|--------|---|
| RM-NTZ 98-002 | Adults | Placebo | 25 | 0.061 | 11 (8+3) |
| | | NTZ | 25 | -0.312 | 15 (9+6) |
| RM-NTZ 98-002 | Children (1-11 yrs) | Placebo | 25* | 0.039 | 10 (7+3) |
| | | NTZ | 24 | 0.333 | 5 (4+1) |
| RM02-3007 | Children (< 4 yrs) | Placebo | 22 | 0.096 | 6 (4+2) |
| | | NTZ | 25 | 0.116 | 11 (6+5) |
| RM02-3008 | Children (1-11 yrs) | Placebo | 25 | 0.311 | 5 (3+2) |
| | | NTZ | 25 | 0.250 | 4 (1+3) |

* One patient excluded by the sponsor from analyses of clinical response is included in the calculation of kappa.

The negative Kappa for the NTZ arm in adults population in Study RM-NTZ-98-002 raises questions about meaningfulness of parasitological response as an efficacy endpoint. This together with statistically insignificant clinical response of NTZ compared to placebo for this population leads one to conclude that evidence of efficacy of NTZ for adults is not adequate.

Further analyses for the subgroups based on age group in children, gender and patients with only *C. Parvum* infection is contained in Section 3.3.

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3.2 DIARRHEA CAUSED BY *GIARDIA LAMBLIA*

3.2.1 STUDY DESIGN

The sponsor conducted two randomized studies to evaluate efficacy of NTZ in the treatment of diarrhea caused by *G. Lambli*a. Dosing and study design information is given in Table 1.

Study RM-NTZ-98-001 was a double-blind placebo-controlled study in adults (age 12-65 years). To be eligible for inclusion in the study, patients must have reported diarrhea (> 4 unformed bowel movements/day) and cysts or trophozoites of *Entamoeba histolytica/dispar* or *Giardia Lambli*a must have been identified in a stool sample collected within 7 days prior to enrollment. The sample size for study RM-NTZ-98-001 was calculated (with an estimated 40/60 distribution of patients with amoebiasis or giardiasis and 7% drop out rate) to provide 90% power to detect a difference between an 80% response rate for the active treatment group and a 25% response rate for the placebo group using a two sided alpha of 0.05. Upon enrollment, the patients were randomized to either NTZ or Placebo arms, and received 3 consecutive days of treatment. Patients with bacterial causes of diarrhea were excluded from the analysis of clinical response. However, patients with other intestinal parasitic infections were not excluded from the study, due to high prevalence of intestinal parasitic infections in the Nile Delta Region and the potential difficulties in enrolling patients infected by *G.Lambli*a alone. This study was terminated early so that the principle investigator could take a planned leave of absence.

Study RM-NTZ-99-010 was single-blind (parasitologist blinded) active controlled study in children (age 2-11 years). Metrodidazole was used as control treatment. To be eligible for inclusion in the study, patients must have reported acute diarrhea (> 2 unformed bowel movements/day) or chronic diarrhea (unformed stools with or without increased frequency for more than 4 weeks) and positive immuno-assay for *Giardia Lambli*a in a stool sample collected within 7 days prior to enrollment. The sample size for study RM-NTZ-99-010 was calculated (with an estimated 8% drop out rate) to provide 80% power to detect a less than 20% difference between response rates for the active treatment group and the active control group using a two sided alpha of 0.05. It was assumed that response rates for both treatment groups were equal to 85% for this sample size calculation. Patients with stool sample positive for *Entamoeba histolytica* or *C. Parvum* were not enrolled in the study. Patients with bacterial causes of diarrhea were excluded from the analysis of clinical response. Parasitological diagnosis was reconfirmed at baseline in this study and patients for whom the diagnosis was not confirmed were to be excluded from efficacy analyses.

A post-treatment physical exam was performed on day 7 (\pm 2 days). Two post-treatment fecal samples were obtained from each patient between day 7 and 10 (\pm 2 days). Each fecal sample was subjected to a parasitological exam. The criteria for evaluating clinical (Well, Continuing Illness, Clinical Failure) and parasitological (Eradication, Persistence) responses were clearly stated in the protocol.

3.2.2 EFFICACY ENDPOINTS

All three studies evaluated two efficacy endpoints: **clinical response** (well, continuing illness, clinical failure) on study day 7 (4 days after the end of treatment) and **parasitological response** (eradication or persistence) based on two stool samples collected between study days 7 and 10. For the clinical endpoint, 'well' response was defined as 'no symptoms, no watery stools and no more than 2 soft stools with no hematochezia within the past 24 hours' or 'no symptoms and no unformed stools within the past 48 hours.' For the parasitological endpoint, 'Eradication' was defined as 'no cysts of the parasite observed in either of the 2 post-treatment parasitological examinations'.

In Study RM-NTZ-98-001, both of these endpoints were stated as primary endpoints, whereas in Study RM-NTZ-99-010 clinical response was stated as primary endpoint and parasitological response was stated as secondary endpoint.

Time from initiation of treatment to passage of last unformed stool (as reported by the patient in a diary) was evaluated as secondary efficacy endpoint in both studies.

In Study RM-NTZ-99-010, "Therapeutic Response" defined as simultaneous "Well" clinical response and "eradicated" parasitological response was also evaluated as a secondary endpoint.

Reviewer's Comment

Since the sponsor is seeking indications of "Treatment of Diarrhea" — , both clinical response and parasitological response are treated as primary endpoints in this review.

3.2.3 STATISTICAL METHODOLOGY USED BY THE SPONSOR

Fisher's exact tests were used to compare the treatment groups in terms of clinical and parasitological response rates. The median time from initiation of therapy to date of last unformed stool was compared for the two treatment groups using a median test.

Reviewer's Comment

Median Test is a chi-squared test applied to the 2×2 contingency table constructed in such a way that the two entries in the first column are the number of observations for treatment 1 (say placebo) that are above and below the grand median (the median of all observations the both treatment groups combined), and the two entries in the second column are the number of observations for treatment 2 (say NTZ) that are above and below the grand median. For more discussion of this test the reader is referred to the reference (4).

3.2.4 DIFFERENCES/SIMILARITIES IN THE TWO STUDIES

The following table shows differences/similarities among the two studies in terms of location, study design, patient population and other characteristics.

Table 9: Controlled Clinical Trials for *G. Lamblia*

| | RM-NTZ-98-001 | RM-NTZ-98-010 |
|--|--|---|
| Center | Egypt | Peru |
| Patient population | Adults (age ≥ 12 years) with Ameobiasis or Giardiasis | Children (age 2-11 years) with Giardiasis only. |
| Control Treatment | Placebo | Metrodinazole |
| Blind | Double-blind | Parasitologist blinded |
| Diarrhea | > 4 unformed stools /day | ≥ 3 unformed stools per day OR unformed stools with or without increased frequency for more than 4 weeks. |
| Parasitological diagnosis | Not confirmed at Baseline (enrollment) | Confirmed at Baseline (enrollment) |
| Excluded from efficacy analyses | Patients with Bacterial infections | 1> Patients with bacterial infections 2> Patients with no cysts at baseline |

3.2.5 PATIENT DISPOSITION

The following table gives an overview of the patient disposition including drop-outs and protocol deviations for both studies.

Table 10: Patient disposition for Controlled clinical trials for *G. Lamblia*

| | RM-NTZ-98-001 | | RM-NTZ-99-010 | |
|---|----------------------|----------------|----------------------|----------------------|
| | NTZ | Placebo | NTZ | Metronidazole |
| ENROLLED | 48 | 45 | 55 | 55 |
| COMPLETED | 47 | 41 | 55 | 53 |
| WITHDRAWN | | | | |
| Withdrew Consent | 1 | 1 | 0 | 0 |
| Loss to follow-up | 0 | 1* | 0 | 2* |
| Error in Diagnosis | 0 | 1 | 0 | 0 |
| Error in Diagnosis and Loss to Follow-up | 0 | 1 | 0 | 0 |
| No baseline parasite | Not Tested | | 0 | 0 |
| Death | 0 | 1 | 0 | 0 |
| OTHER INFECTIONS | | | | |
| Parasitic | 9 | 8 | 11 | 8 |
| PROTOCOL DEVIATIONS | | | | |
| Concomitant Medication | 0 | 0 | 1 | 0 |
| Late or No show for Post Rx exam Day 7 | 5 | 4 | 1 | 0 |
| *Patients treated as failures for efficacy analyses | | | | |

3.2.6 DEMOGRAPHICS AND BASELINE CHARACTERISTICS

The sponsor summarized demographic and disease related characteristics for all the subjects in each study by their treatment assignment. These were generally comparable across the treatment groups.

Table 11: Demographics and Baseline Characteristics

| Demographic or Disease Related Characteristic | STUDY | | | |
|---|---------------|-------------|---------------|---------------|
| | RM-NTZ-98-001 | | RM-NTZ 99-010 | |
| | NTZ | Placebo | NTZ | Metronidazole |
| Male | 31 | 26 | 27 | 27 |
| Female | 16 | 16 | 28 | 28 |
| Age (yrs)- Mean (s.d.) | 34.5 (17.7) | 29.9 (14.4) | 5.51 (2.53) | 5.84 (2.61) |
| Weight (kgs)- Mean (s.d.) | 63.9 (16.6) | 58.4 (18.6) | 21.45 (7.86) | 20.87 (5.76) |
| Stool Frequency | | | | |
| 3-10 stools /day | 41 | 36 | 53 | 55 |
| > 10 stools /day | 5 | 6 | 2 | 0 |
| Stool Consistency | | | | |
| Liquid | 16 | 16 | 23 | 15 |
| Semisolid | 31 | 26 | 30 | 38 |
| Liquid & Semisolid | 0 | 0 | 2 | 2 |
| Duration of Diarrhea-Median no. of days | 9 | 9 | 13 | 18 |
| Abdominal abnormalities | 36 | 32 | 17 | 19 |

For study RM-NTZ-98-001, the sponsor provided demographic results for each of the two subgroups, Ameobiasis patients, and Giardiasis patients. Again these were generally comparable across the two treatment groups.

3.2.7 SPONSOR'S EFFICACY RESULTS

3.2.7.1 Study RM-NTZ-98-001

Out of 93 patients enrolled in the study, two patients decided not to participate in the study and returned all of the study medication. These patients were excluded from all efficacy analyses conducted by the sponsor. Also the sponsor excluded two patients (patient numbers 81 and 83) who were enrolled without diagnosis of amoebiasis or giardiasis from all efficacy analysis. The following table shows sponsor's primary analyses for the clinical and parasitological response using Fisher's exact tests for the Intent-To-Treat (ITT) population. The sponsor stated that precise median time to last unformed stool for the placebo group

could not be determined since patients were not followed up past the date of post-treatment examination (day 6).

Table 12. Sponsor's Efficacy results for Study RM-NTZ-98-001 – ITT population

| Study Medication | "Well" Clinical Response | "Eradicated" Parasitological Response | Median time to last unformed stool |
|----------------------------|--------------------------|---------------------------------------|------------------------------------|
| AMOEBIASIS PATIENTS | | | |
| NTZ | 28/36 (78%) | 25/36 (69%) | 3 days |
| Placebo | 13/31 (42%) | 12/31 (39%) | 6 days |
| p-value (two sided) | .0052 | .0148 | .0012 (Median test) |
| GIARDIASIS PATIENTS | | | |
| NTZ | 14/17 (82%) | 12/17 (71%) | 3 days |
| Placebo | 6/19 (32%) | 0/19 (0%) | 6 days |
| p-value (two sided) | .0031 | <.0001 | .0068 (Median test) |

Reviewer's Comments:

- 1> *In this study, 17 Giardiasis patients (9-NTZ, 8-placebo) had other additional infections besides Giardiasis. When patients infected with only Giardia Lamblia infection were isolated, the sample size was reduced to 18 patients, of whom, only 8 were randomized to NTZ (just one patient out of 8 was female). These sample sizes are very small and hence the results of this study, although statistically significant, can not be generalized.*
- 2> *This reviewer also investigated the correlation between clinical and parasitological response (See Sections 3.2.8 and 3.3) and the subgroup of patients with only Giardiasis (and not Amoebiasis) (See Section 3.3.4).*

3.2.7.2 Study RM-NTZ-99-010

The following table shows sponsor's primary analyses for the clinical and parasitological response using Fisher's exact tests for the Intent-To-Treat (ITT) population (consisting of all 110 patients enrolled in the study). The 95% confidence interval for the difference is calculated with correction for continuity. The time to last unformed stool was compared using Median Test. In this study the sponsor also analyzed "therapeutic" response defined as simultaneous occurrence of both "well" clinical response and "eradicated" parasitological response.

Table 13. Sponsor's Efficacy results for Study RM-NTZ-99-010 – ITT population

| Study Medication | “Well” Clinical Response | “Eradicated” Parasitological Response | Therapeutic Response | Median Time to last unformed stool (No. of patients with data) |
|----------------------------|--------------------------|---------------------------------------|----------------------|---|
| CHILDREN | | | | |
| NTZ | 47/55 (85%) | 39/55 (71%) | 36/55 (65%) | 4 days (23/55) |
| Metronidazole | 44/55 (80%) | 41/55 (75%) | 35/55 (64%) | 4 days (12/55) |
| p-value (two sided) | .6148 | .8307 | 1.000 | .3799 (Median test) |
| Difference | 5% | 4% | 1% | |
| 95% CI | (-10% , 21%) | (-21%, 14%) | (-17%, 20%) | |

Reviewer's Comments:

- 1> *The results in the above table support the sponsor's claim that clinical response rate for NTZ is no more than 20% inferior to that of metronidazole. However, since the confidence interval for the difference in parasitological response rates includes a lower bound of -20%, it does not provide sufficient evidence of non-inferiority of NTZ compared to metronidazole in terms of parasitological response.*
- 2> *The sponsor also conducted “per protocol” analyses for the subset of patients who took all their study medication and returned for follow-up. For these analyses 17 patients were excluded from the ITT population for protocol violations(8 –NTZ, 9-Metronidazole). These results were consistent with those for the ITT population in the above table.*
- 3> *This reviewer conducted several analyses to evaluate the evidence based on gender, and patients with only G. Lamblia infection. (See Section 3.3). Also this reviewer investigated the correlation between clinical and parasitological response extensively (See Sections 3.2.8 and 3.3).*

3.2.8 CORRELATION BETWEEN CLINICAL RESPONSE AND PARASITOLOGICAL RESPONSE

Multiple endpoints are often used in clinical trials to characterize drug benefit in several ways or to characterize different aspects of drug benefit. These endpoints may be correlated weakly or strongly. Kappa Coefficient is a quantitative measure of reproducibility of drug benefit measured with two nominal endpoints. Theoretical Range of Kappa is [-1, 1]. The use of kappa to evaluate the correlation between clinical and parasitological response is discussed in Section 3.4.

In this application, for the NTZ arm, positive kappa values are expected in order to consider parasitological response to be a meaningful endpoint. Kappa Coefficients for all the two Giardia studies are provided in the following table.

Table 14: Kappa Coefficients

| Study | Population | Treatment | N | Kappa | No. of discordant pairs (well & persistence + ill & eradicated) |
|-----------------------|------------------------|---------------|----|-------|---|
| RM- NTZ- 98-001 | Adults (Giardiasis) | NTZ | 17 | -.283 | 8 (5+3) |
| | | Placebo | 19 | N/A | 6 (6+0) |
| RM- NTZ- 99-010 | Children (2-11 yrs) | NTZ | 55 | .276 | 14 (11+3) |
| | | Metronidazole | 55 | .227 | 15 (9+6) |

The negative Kappa for the NTZ arm in adults population in Study RM-NTZ-98-001 raises questions about meaningfulness of parasitological response as an efficacy endpoint. This together with small sample size of the group of patients with Giardiasis only (11-NTZ, 11-placebo) leads one to conclude that evidence of efficacy of NTZ for adults with Giardiasis is not adequate.

Further analyses for the subgroups based on age group in children, gender and patients with only *G. Lamblia* infection is contained in Section 3.3.

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3.3 FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

In this section we evaluate findings in subgroup/special populations for studies RM-NTZ-98-002 and RM02-3007 in patients with *C. Parvum* infection and for studies RM-NTZ-98-001 and RM-NTZ-99-010 in patients with *Giardia Lamblia* infection. These analyses are post-hoc and the sample sizes of some of the subgroups are small resulting in substantial decrease in power to detect treatment differences.

Study RM02-3008 in patients with *C. Parvum* infection failed to show any evidence of drug benefit. Therefore subgroup analyses were not carried out for this study. Study RM-NTZ-98-001 had a very small sample of patients with *Giardia Lamblia* infection. Therefore subgroup analyses based on gender was not carried out for this study.

3.3.1 STUDY RM-NTZ-98-002

The following tables show the reviewer's results for the subgroups of the ITT population based on gender and age group of children.

Table 15: Adult ITT population Subgroups in Study RM-NTZ-98-002

| Study Medication | "Well" Clinical Response | "Eradicated" Parasitological Response | Kappa (s.d.) | No of discordant pairs (Well& Persistence+ Ill & Eradicated) |
|------------------------|--------------------------|---------------------------------------|---------------|--|
| ADULTS | | | | |
| NTZ | 18/25 (72%) | 15/25 (60%) | -0.316 (.162) | 15 (9+6) |
| Placebo | 11/25 (44%) | 6/25 (24%) | 0.061 (.182) | 11 (8+3) |
| p-value (two sided) | .0845 | .0209 | | |
| ADULTS – FEMALE | | | | |
| NTZ | 11/13 (84.6%) | 7/13 (53.9%) | -0.3 (.181) | 8 (6+2) |
| Placebo | 4/10 (40%) | 2/10 (20 %) | 0.091 (.288) | 4 (3+1) |
| p-value (two sided) | .0393 | .1968 | | |
| ADULTS – MALE | | | | |
| NTZ | 7/12 (58.3%) | 8/12 (66.7%) | -0.235 (.264) | 7 (3+4) |
| Placebo | 7/15 (46.7%) | 4/15 (26.7%) | 0.037 (.236) | 7 (5+2) |
| p-value (two sided) | .7036 | .0574 | | |

These analyses show a consistent trend of numerically higher percents for the NTZ arm compared to the placebo arm. However, the differences generally are not statistically significant. Moreover, kappa coefficient is consistently negative for the NTZ arm raising doubts about the meaningfulness of one of the two endpoints. Efficacy of NTZ has not been adequately demonstrated for the population of adults.

Table 16: Children ITT * population Subgroups in Study RM-NTZ-98-002

| Study Medication | “Well” Clinical Response | “Eradicated” Parasitological Response | Kappa (s.d.) | No of discordant pairs (Well& Persistence+ Ill & Eradicated) |
|------------------------------|--------------------------|---------------------------------------|--------------|--|
| CHILDREN | | | | |
| NTZ | 21/24 (87.5%) | 18/24 (75%) | .333 (.262) | 5 (4+1) |
| Placebo | 9/25 (36%) | 5/25 (20%) | .039 (.188) | 10 (7+3) |
| p-value (two sided) | .0003 | .0002 | | |
| CHILTDREN – FEMALE | | | | |
| NTZ | 12/12 (100%) | 9/12 (75%) | N/A | 3 (3+0) |
| Placebo | 5/9 (55.6%) | 2/9 (22.2%) | -.047 (.262) | 5 (4+1) |
| p-value (two sided) | .0211 | .03 | | |
| CHILDREN – MALE | | | | |
| NTZ | 9/12 (75%) | 9/12 (75%) | .556 (.278) | 2 (1+1) |
| Placebo | 4/16 (25%) | 3/16 (18.8%) | .091 (.262) | 5 (3+2) |
| p-value (two sided) | .02 | .0061 | | |
| CHILDREN – AGE < 4 | | | | |
| NTZ | 10/11 (90.9%) | 8/11 (72.7%) | -.158 (.128) | 4 (3+1) |
| Placebo | 4/11 (36.4%) | 3/11 (27.3%) | .377 (.29) | 3 (2+1) |
| p-value (two sided) | .024 | .086 | | |
| CHILDREN – AGE 4-11 | | | | |
| NTZ | 11/13 (84.6%) | 10/13 (76.9%) | .755 (.228) | 1 (1+0) |
| Placebo | 5/14 (35.7%) | 2/14 (14.3%) | -.256 (.144) | 7 (5+2) |
| p-value (two sided) | .018 | .002 | | |

* Includes one child in the placebo arm who was excluded from analysis of clinical response by the sponsor due to bacterial cause of diarrhea.

For the population of children, statistically significant higher percent of response is observed for the NTZ arm compared to the placebo arm. Also kappa coefficients are consistently positive (with the exception of the subgroup of children < 4 years of age). Efficacy of NTZ is adequately demonstrated for the population of children in this study.

In this study, 21 patients had mixed infections of whom 13 were children (4 placebo, 9 NTZ) and 8 were adults (4 placebo, 4 NTZ). Since these patients may potentially introduce bias, this reviewer conducted sensitivity analyses excluding these patients from the ITT population. The following tables show that the efficacy results for this population of patients

with only *C. Parvum* infection and for the subgroups of this population are consistent with the results for the ITT population and its subgroups.

Table 17: Adult *C. Parvum*-only population subgroups in Study RM-NTZ-98-002

| Study Medication | “Well” Clinical Response | “Eradicated” Parasitological Response | Kappa (s.d.) | No of discordant pairs (Well& Persistence+ Ill & Eradicated) |
|------------------------|--------------------------------|---|---------------|---|
| ADULTS | | | | |
| NTZ | 15/21 (71.4%) | 12/21 (57.1%) | -0.319 (.181) | 13 (8+5) |
| Placebo | 9/21 (42.9%) | 6/21 (28.6%) | 0.087 (.209) | 9 (6+3) |
| p-value (two sided) | .118 | .118 | | |
| ADULTS – FEMALE | | | | |
| NTZ | 11/13 (84.6%) | 7/13 (53.9%) | -0.3 (.181) | 8 (6+2) |
| Placebo | 3/8 (37.5%) | 2/8 (25 %) | 0.143 (.347) | 3 (2+1) |
| p-value (two sided) | .056 | .367 | | |
| ADULTS – MALE | | | | |
| NTZ | 4/8 (50%) | 5/8 (62.5%) | -0.25 (.332) | 5 (2+3) |
| Placebo | 6/13 (46.2%) | 4/13 (30.8%) | 0.049 (.264) | 6 (4+2) |
| p-value (two sided) | 1.0 | .203 | | |

Table 18: Children *C. Parvum*-only population subgroups in Study RM-NTZ-98-002

| Study Medication | “Well” Clinical Response | “Eradicated” Parasitological Response | Kappa (s.d.) | No of discordant pairs (Well& Persistence+ Ill & Eradicated) |
|------------------------------|--------------------------------|---|---------------|---|
| CHILDREN | | | | |
| NTZ | 13/15 (86.7%) | 11/15 (73.3%) | 0.189 (.270) | 4 (3+1) |
| Placebo | 6/21 (28.6%) | 4/21 (19.1%) | -0.037(.204) | 8 (5+3) |
| p-value (two sided) | .0008 | .0019 | | |
| CHILTDREN – FEMALE | | | | |
| NTZ | 8/8 (100%) | 6/8 (75%) | N/A | 2 (2+0) |
| Placebo | 4/8 (50%) | 2/8 (25%) | 0.0 (.306) | 4 (3+1) |
| p-value (two sided) | .0769 | .1319 | | |
| CHILDREN – MALE | | | | |
| NTZ | 5/7 (71.4%) | 5/7 (71.4%) | 0.3 (.391) | 2 (1+1) |
| Placebo | 2/13(15.4%) | 2/13(15.4%) | -0.182 (.089) | 4 (2+2) |
| p-value (two sided) | .0223 | .0223 | | |
| CHILDREN – AGE < 4 | | | | |
| NTZ | 6/7 (85.7%) | 4/7 (57.1%) | -0.273 (.230) | 4 (3+1) |
| Placebo | 2/9 (22.2%) | 2/9 (22.2%) | 0.357 (.367) | 2 (1+1) |
| p-value (two sided) | .041 | .302 | | |
| CHILDREN – AGE 4-11 | | | | |
| NTZ | 7/8 (87.5%) | 7/8 (87.5%) | 1.0 (N/A) | 0 (0+0) |

| | | | | |
|----------------------------|--------------|--------------|---------------|---------|
| Placebo | 4/12 (33.3%) | 2/12 (16.7%) | -0.286 (.150) | 6 (4+2) |
| p-value (two sided) | .028 | .005 | | |

3.3.2 STUDY RM02-3007

Out of 50 patients enrolled in this study, 3 patients (all in placebo group, all male) with no oocysts of *C. Parvum* in the stool sample collected at baseline were excluded by the sponsor from all efficacy analyses. Since these patients were treated and the clinical response and parasitological response was observed for all three patients, this reviewer conducted sensitivity analyses by including these patients. One may recall that in the other similarly designed study (RM-NTZ-98-002) in HIV negative children with *C. Parvum* infection, patients were not tested for oocysts of *C. Parvum* at baseline, and therefore no one was excluded from that study due to absence of oocysts of *C. Parvum* at baseline.

The following are the results of the reviewer's sensitivity analyses.

Table 19. Efficacy results for Study RM02-3007 – All Patients

| Study Medication | “Well” Clinical Response | “Eradicated” Parasitological Response | Kappa (s.d.) | No of discordant pairs (Well& Persistence+ Ill & Eradicated) |
|----------------------------|---------------------------------|--|---------------------|---|
| CHILDREN | | | | |
| NTZ | 14/25 (56%) | 13/25 (52%) | 0.116 (.198) | 11 (6+5) |
| Placebo | 8/25 (32%) | 6/25 (24%) | 0.409 (.198) | 6 (4+2) |
| p-value (two sided) | .1536 | .0792 | | |
| CHILDREN- FEMALE | | | | |
| NTZ | 7/9 (77.8%) | 6/9 (66.7%) | 0.182 (.336) | 3 (2+1) |
| Placebo | 1/7 (14.3%) | 0/7 (0%) | N/A | 1 (1+0) |
| p-value (two sided) | .0406 | .0114 | | |
| CHILDREN – MALE | | | | |
| NTZ | 7/16 (43.8%) | 7/16 (43.8) | -0.016 (.25) | 8 (4+4) |
| Placebo | 7/18 (38.9%) | 6/18 (33.3%) | 0.4 (.222) | 5 (3+2) |
| p-value (two sided) | 1.0 | .7254 | | |

In these analyses, statistical significance is not reached for both endpoints for the population of all children as well as for the subgroup of male children.

In the sponsor's ITT analyses in Table 6, statistical significance is reached for both endpoints for the ITT population of all children. However, when these analyses are carried out for the gender based subgroups of ITT population, the statistical significance is reached

for the subgroup of female children, but not for the subgroup of male children (consistent with Table 17).

3.3.3 STUDIES RM-NTZ-98-002 AND RM02-3007 POOLED

This reviewer analyzed the pooled data on all children to evaluate the collective evidence for children with *C. Parvum* infection. The pooling of data was done under certain restrictions suggested by the medical officer reviewing this application. These restrictions are as follows:

- 1> Children who had other infections in addition to *C. Parvum* infection and/or did not take the medication were excluded
- 2> All children whose stool samples tested positive for oocysts of only *C. Parvum* at the screening visit were included.

The results of these analyses are as follows:

Table 20: Children *C. Parvum*-only population – Pooled data

| Study Medication | “Well” Clinical Response | “Eradicated” Parasitological Response | Kappa (s.d.) | No of discordant pairs (Well& Persistence+ Ill & Eradicated) |
|---|--------------------------------|---|---------------|---|
| ALL DATA | | | | |
| Placebo | 14/46 (30.4%) | 10/46 (21.7%) | 0.218 (.153) | 14 (9+5) |
| NTZ | 26/39 (66.7%) | 23/39 (59.0%) | 0.182 (.158) | 15 (9+6) |
| p-value (two sided) | .0011 | .0007 | | |
| AGE < 4 YEARS | | | | |
| Placebo | 10/34 (29.4%) | 8/34 (23.5%) | 0.398 (.175) | 8 (5+3) |
| NTZ | 19/31 (61.3%) | 16/31 (51.6%) | 0.025 (.176) | 15 (9+6) |
| p-value (two sided) | .0131 | .0231 | | |
| AGE 4-11 YEARS (only in Study RM-NTZ-98-002) | | | | |
| Placebo | 4/12 (33.3%) | 2/12 (16.7%) | -0.286 (.15) | 6 (4+2) |
| NTZ | 7/8 (87.5%) | 7/8 (87.5%) | 1.0 (N/A) | 0 (0+0) |
| p-value (two sided) | .0281 | .0045 | | |
| FEMALE | | | | |
| Placebo | 5/15 (33.3%) | 2/15 (13.3%) | 0.118 (.236) | 5 (4+1) |
| NTZ | 14/16 (87.5%) | 11/16 (68.8%) | 0.130 (.235) | 5 (4+1) |
| p-value (two sided) | .0032 | .0032 | | |
| MALE | | | | |
| Placebo | 9/31 (29.0%) | 8/31 (25.8%) | 0.271 (.188) | 9 (5+4) |
| NTZ | 12/23 (52.2%) | 12/23 (52.2%) | 0.129 (.207) | 10 (5+5) |
| p-value (two sided) | .0994 | .0861 | | |
| AFRICAN – STUDY RM02-3007 | | | | |
| Placebo | 8/25 (32%) | 6/25 (24%) | 0.409 (.198) | 6 (4+2) |
| NTZ | 13/24 (54.2%) | 12/24 (50%) | 0.083 (.203) | 11 (6+5) |
| p-value (two sided) | .1536 | .0792 | | |
| CAUCASIAN – STUDY RM-NTZ-98-002 | | | | |
| Placebo | 6/21 (28.6%) | 4/21 (19.1%) | -0.037 (.204) | 8 (5+3) |

| | | | | |
|----------------------------|---------------|---------------|--------------|---------|
| NTZ | 13/15 (86.7%) | 11/15 (73.3%) | 0.189 (.270) | 4 (3+1) |
| p-value (two sided) | .0008 | .0019 | | |

These results show a consistent clinical response rate for placebo ranging from 28.6% to 33.3%. However the clinical response rate for NTZ is highly variable ranging from 52.2% to 87.5%. Parasitological response rate varies greatly for both placebo as well as NTZ. Correlation between clinical and parasitological response rates as measures by kappa coefficient is inconsistent. For those subgroups, where statistical significance is not reached, the trend in both clinical and parasitological response is in favor of the test drug NTZ. In summary, efficacy of NTZ in children with *C. Parvum* infection is demonstrated in terms of clinical response endpoint, but there is lack of robustness in terms of parasitological response endpoint.

3.3.4 STUDY RM-NTZ-98-001

In this study, patients (age ≥ 12 years) were enrolled with diagnosis of either Amoebiasis or Giardiasis or both. Since the sponsor is seeking indication of Giardiasis only, the reviewer obtained the following results for the subgroups based on the type of infection.

Table 21: Adult population subgroups in Study RM-NTZ-98-001

| Study Medication | “Well” Clinical Response | “Eradicated” Parasitological Response | Kappa (s.d.) | No of discordant pairs (Well & Persistence+ Ill & Eradicated) |
|--------------------------------|--------------------------------|---|-----------------|--|
| ONLY GIARDIASIS | | | | |
| Placebo | 4/11 (36.4%) | 0/11 (0%) | N/A | 4 (4+0) |
| NTZ | 10/11 (90.9%) | 9/11 (81.8%) | -.138 (.100) | 3 (2+1) |
| p-value (two sided) | .024 | .0002 | | |
| GIARDIASIS + AMOEBIASIS | | | | |
| Placebo | 2/8 (25.0%) | 0/8 (0%) | N/A | 2 (2+0) |
| NTZ | 4/6 (66.7%) | 3/6 (50%) | -.667 (.287) | 5 (3+2) |
| p-value (two sided) | .287 | .055 | | |
| ALL GIARDIASIS | | | | |
| Placebo | 6/19 (31.6%) | 0/19 (0%) | N/A | 6 (6+0) |
| NTZ | 14/17 (82.4%) | 12/17 (70.6%) | -.283 (.115) | 8 (5+3) |
| p-value (two sided) | .003 | <.0001 | | |

Although statistically significant clinical and parasitological response is demonstrated, the sample size is very small for the subgroup of patient with only Giardiasis and not Amoebiasis (11 treated with NTZ, only 3 female). Also 17 patients (9-NTZ, 8-placebo) had other additional infections besides Giardiasis. In this study, when patients infected with only *Giardia Lambli*a infection were isolated, the sample size was reduced to 18 patients, of whom,

only 8 were randomized to NTZ (just one patient out of 8 was female). These sample sizes were very small and hence the results of this study, although statistically significant, could not be generalized. In addition kappa coefficients for all NTZ arms are negative implying poor correlation between clinical and parasitological endpoints. Thus, efficacy of NTZ for the population of adults with Giardiasis has not been adequately demonstrated.

3.3.5 STUDY RM-NTZ-99-010

In this study in children conducted at a single center in Peru, 110 patients were enrolled of whom 25 patients (11- Metronidazole, 14 -NTZ) were 2-3 years of age and 85 patients (44 - Metronidazole, 41-NTZ) were 4-11 years of age. All patients were diagnosed with Giardiasis re-confirmed at baseline. ITT population consisted of all 110 patients. The following table shows the results of the reviewer's analyses for the subgroups of ITT population based on the age group and gender. The continuity correction is not used in the calculation of 95% confidence intervals.

Table 22: Children ITT population Subgroups in Study RM-NTZ-99-010

| Study Medication | "Well" Clinical Response | "Eradicated" Parasitological Response | Kappa (s.d.) | No of discordant pairs (Well& Persistence+ Ill & Eradicated) |
|------------------------------|--------------------------|---------------------------------------|--------------|--|
| ALL CHILDREN | | | | |
| Metronidazole | 44/55 (80%) | 41/55 (74.6%) | .227 (.147) | 15 (9+6) |
| NTZ | 47/55 (85.5%) | 39/55 (70.9%) | .276 (.139) | 14 (11+3) |
| 95% CI* | (-8.64, 19.54) | (-20.3, 13.0) | | |
| CHILDREN – FEMALE | | | | |
| Metronidazole | 23/28 (82.1%) | 21/28 (75%) | .368 (.206) | 6 (4+2) |
| NTZ | 25/28 (89.3%) | 19/28 (67.7%) | .404 (.173) | 6 (6+0) |
| 95% CI* | (-11.04, 25.44) | (-30.91, 16.31) | | |
| CHILDREN – MALE | | | | |
| Metronidazole | 21/27 (77.8%) | 20/27 (74.1%) | .09 (.201) | 9 (5+4) |
| NTZ | 22/27 (81.5%) | 20/27 (74.1%) | .15 (.205) | 8 (5+3) |
| 95% CI* | (-17.75, 25.15) | (-23.37, 23.37) | | |
| CHILDREN – AGE < 4 | | | | |
| Metronidazole | 9/11 (81.8%) | 9/11 (81.8%) | .389 (.353) | 2 (1+1) |
| NTZ | 13/14 (92.9%) | 9/14 (64.3%) | .243 (.209) | 4 (4+0) |
| 95% CI* | (-15.44, 37.52) | (-51.43, 16.37) | | |
| CHILDREN – AGE 4-11 | | | | |
| Metronidazole | 35/44 (79.6%) | 32/44 (72.7%) | .192 (.161) | 13 (8+5) |
| NTZ | 34/41 (82.9%) | 30/41 (73.2%) | .298 (.168) | 10 (7+3) |
| 95% CI* | (-13.19, 19.95) | (-18.46, 19.34) | | |

* 95% Confidence Interval for the difference (NTZ-Metronidazole) - continuity correction not used

The above analyses show that for all the subgroups, the non-inferiority of NTZ compared to metronidazole with a margin of 20% is demonstrated with 95% confidence in terms of the clinical response rates, but not in terms of the parasitological response rates.

These results for the subgroups are consistent with those for the ITT population. This reviewer also carried out analyses for the "Per protocol" population (17 patients excluded: 8-NTZ, 9-Metronidazole) and for the population of patients with no infections other than infection with *Giardia Lambli*a (19 patients excluded for mixed infections: 11-NTZ, 8-Metronidazole). These results (not shown here) were also consistent with those for the ITT population.

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3.4 STATISTICAL AND TECHNICAL ISSUES

3.4.1 KAPPA COEFFICIENT

Multiple endpoints are often used in clinical trials to characterize drug benefit in several ways or to characterize different aspects of drug benefit. These endpoints may be correlated weakly or strongly. Kappa Coefficient is a quantitative measure of reproducibility of drug benefit measured with two nominal endpoints. Theoretical Range of Kappa is [-1, 1]. In statistical literature, a guideline for assessing agreement between two endpoints has been published (2) and is as follows: For $0 \leq \text{kappa} < 0.4$, there is at best marginal agreement, for $0.4 \leq \text{kappa} < 0.75$, the agreement is good and for $\text{kappa} \geq 0.75$, there is excellent agreement between the two endpoints.

In this section, we discuss the use of Kappa to evaluate correlation between clinical response and parasitological response. The following table shows concordant and discordant pairs of responses.

Table 23: Concordance and Discordance

| | Clinical Response | | |
|--------------------------|--------------------|----------------------------|--|
| Parasitological Response | <i>Eradicated</i> | <i>Well</i> Concordance | <i>Continuing Illness</i> Discordance |
| | <i>Persistence</i> | Discordance | Concordance |

Kappa close or equal to +1.0 implies that most patients experiencing “well” clinical response are also experiencing “Eradicated” parasitological response or vice a versa (that is most patients in “Continuing Illness” clinical response category are also in “persistence” parasitological response category). In this case, the number of discordant pairs is close to zero and both clinical response and parasitological response are equally effective in assessing drug benefit, and either one would suffice as a primary endpoint for evaluation of efficacy.

Table 24: Hypothetical Example of Kappa = 1.0

| DRUG A | Clinical Response | | | |
|--------------------------|--------------------|-------------------|--------------------------------|--------------------------|
| Parasitological Response | <i>Eradicated</i> | <i>Well</i> 10 | <i>Continuing Illness</i> 0 | <i>Total</i> 10 (40%) |
| | <i>Persistence</i> | 0 | 15 | 15 |
| | <i>Total</i> | 10 (40%) | 15 | 25 |

Kappa close or equal to 0.0 implies that the two endpoints are independent. That is probability of “well” clinical response given “eradicated” parasitological response and probability of “well” clinical response given “persistence” parasitological response are same and equal the overall (unconditional) probability of “well” clinical response. In this case the

number of concordant pairs is approximately same as the number of discordant pairs, and simultaneous (joint) evaluation both endpoints may be needed to assess the drug benefit.

Table 25: Hypothetical Example of Kappa = 0.0

| DRUG B | Clinical Response | | | Total |
|--------------------------|--------------------|-------------|---------------------------|----------|
| | | <i>Well</i> | <i>Continuing Illness</i> | |
| Parasitological Response | <i>Eradicated</i> | 4 | 6 | 10 (40%) |
| | <i>Persistence</i> | 6 | 9 | 15 |
| | <i>Total</i> | 10 (40%) | 15 | 25 |

Kappa close or equal to -1.0 implies that most patients experiencing “well” clinical response are in “persistence” parasitological response category and vice a versa. In this case the number of discordant pairs is very high and the drug benefit is not reproducible when assessed through these endpoints. One has to use caution in the evaluation of efficacy of the drug in this situation as one of the two endpoints may be meaningless in the assessment of efficacy.

Table 26: Hypothetical Example of Kappa* $\cong -1.0$

| DRUG C | Clinical Response | | | Total |
|--------------------------|--------------------|-------------|---------------------------|----------|
| | | <i>Well</i> | <i>Continuing Illness</i> | |
| Parasitological Response | <i>Eradicated</i> | 0 | 10 | 10 (40%) |
| | <i>Persistence</i> | 15 | 0 | 15 |
| | <i>Total</i> | 15 (60%) | 10 | 25 |

* exact value of Kappa is - 0.92

For the sake of simplicity, let us disregard the issue of statistical significance and look at the percent rates. From the three tables 24, 25 and 26 above, one can easily see that even though Drug C produced best (60%) clinical response and all three drugs produced same (40%) parasitological response, one can not conclude that Drug C is better than Drugs A and B because of varying patterns of concordance and discordance quantified by Kappa coefficients.

These tables illustrate how difficult it is to interpret the results when varying values of kappa are observed for different treatment arms in the same clinical trial.

For a further discussion, this reviewer recommends an excellent literature review of kappa coefficient published in 2002(3).

3.5 STATISTICAL EVALUATION OF COLLECTIVE EVIDENCE

An overall evaluation of efficacy based on the strength of statistical evidence from each of the five well controlled pivotal studies in this application and a collective assessment of drug performance for all five studies is addressed in the previous sections and is not repeated here. Fully informative tables and text to collectively assess the evidence have also been provided in the previous sections. The overall extent to which study results support the efficacy claim is outlined in Section 3.6 (and also in section 1.1 of Executive summary).

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3.6 CONCLUSIONS AND RECOMMENDATIONS

Single Center Studies

All five studies in this application were designed as single center, single race studies conducted outside of Unites States. This fact seriously compromises the generalizability of the results of these studies to the population in Unites States, and applicability of these results to future non-inferiority studies where NTZ, if approved, may be used as an active comparator. The data from these studies could not be used to assess the effects of important covariates such as race, age group, pathogen, and the interactions of these covariates among each other as these effects were confounded with the study designs and study to study variability.

It is strongly recommended that any future studies in the development of NTZ be designed and conducted as adequate, well-controlled, multi-center studies.

C. Parvum

Adult Studies

Adult HIV negative population (age ≥ 12 years) was studied in only one study, RM-NTZ-98-002, conducted at a single center in Egypt. The difference between NTZ and placebo for the clinical endpoint was not statistically significant. Although statistical significance was demonstrated for the parasitological endpoint, poor correlation between clinical and parasitological endpoints for the NTZ arm ($\kappa = -0.312$) seriously compromises the meaningfulness of parasitological endpoint for this population.

The data in HIV negative adult population did not provide adequate evidence of efficacy of NTZ tablets for the treatment of Diarrhea caused by *Cryptosporidium parvum*. However, the observed trends in the clinical and parasitological response rates were in favor of NTZ.

It is recommended that any future studies be conducted as placebo controlled, multi-center and in patients with only *C. Parvum* infection. An evaluation and interpretation of correlation between clinical and parasitological responses and its implications should be addressed in the data analyses.

Pediatric Studies

The population of HIV negative children (age 1-11 years) was studied in two randomized placebo controlled studies, one, RM-NTZ-98-002, conducted at a single center in Egypt and another, RM02-3007, conducted at a single center in Zambia.

In both studies, the differences between NTZ and placebo were statistically significant (in favor of NTZ) in terms of clinical response (88% versus 38% in Study RM-NTZ-98-002 and 56% versus 23% in Study RM02-3007) and parasitological response (75% versus 20% in Study RM-NTZ-98-002 and 52% versus 14% in Study RM02-3007). However, sensitivity

analyses and subgroup (based on age and gender)/special (*C. Parvum* infection only) population analyses did not, consistently, show statistically significant results.

The data in the population of HIV negative children provided adequate evidence of efficacy of NTZ suspension for the treatment of Diarrhea caused by *C. parvum*,

Another double-blind, placebo-controlled study was conducted in hospitalized, severely malnourished children of age 1-11 years with acquired immune deficiency syndrome (AIDS) in Zambia. In this study, a three-day course of nitazoxanide suspension (100 mg BID in children ages 12-47 months, 200 mg BID in children ages 4 through 11 years) did not produce clinical or parasitological response rates or mortality rates that were significantly different from the placebo control.

Giardia Lamblia

Adult Studies

Adult HIV negative population with *Giardia Lamblia* was studied in only one placebo controlled study, RM-NTZ-98-001, conducted at a single center in Egypt. This study enrolled patients with either giardiasis or amoebiasis or both. The sample sizes were very small and hence the results of this study, although statistically significant, could not be generalized. Also, kappa coefficient for the NTZ arm in the group of patients with giardiasis was negative (-0.283), compromising the meaningfulness of the parasitological endpoint.

The data in HIV negative adult population did not provide adequate evidence of efficacy of NTZ tablets for the treatment of Diarrhea caused by *Giardia Lamblia*

It is recommended that any future studies be conducted as placebo controlled, multi-center and in patients with only *Giardia Lamblia* infection. An evaluation and interpretation of correlation between clinical and parasitological responses and its implications should be addressed in the data analyses.

Pediatric Studies

The population of HIV negative children (age 2-11years) with *Giardia Lamblia* infection was studied in only one active controlled study, RM-NTZ-99-010, conducted at a single center in Peru, where metronidazole was used as an active control. In this study, the non-inferiority of NTZ compared to metronidazole with a margin of 20% was demonstrated with 95% confidence in terms of the clinical response rates (85% for NTZ versus 80% for Metronidazole), but not in terms of the parasitological response rates.

The data in children's population provided adequate evidence of efficacy of NTZ suspension for the treatment of Diarrhea caused by *Giardia Lamblia*.

4 REFERENCES

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Karen Higgins
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