DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
Rockville, MD 20857

Date: February 20, 2002

To: Antiviral Drug Products Advisory Committee Members and Guests

From: Pleconaril Review Team

Through: Debra Birnkrant, MD
Director, DAVDP

Re: Background Package for NDA 21-245: Picovir™ (pleconaril)

I. Summary of Regulatory Issues and Purpose of Meeting

This memorandum serves as an introduction to the FDA presentation at the upcoming March 19, 2002, meeting of the Antiviral Drug Products' Advisory Committee (AVAC). At this meeting we will ask you to discuss the NDA for ViroPharma's Picovir™ (pleconaril) for the treatment of acute picornaviral viral respiratory infection (VRI, "common cold") in adults.

The background materials from FDA represent the findings and opinions of the primary reviewers of each discipline, based on their reviews of the respective submissions from ViroPharma. It must be emphasized that this document represents the review team's preliminary findings, and that no regulatory decision has been made on the status of the application. Indeed, the advice the AVAC provides will be critical in our regulatory decision making.

Pleconaril represents the first antiviral drug to be considered for approval for treatment of the common cold. Currently, only treatments that target individual symptoms of VRI are approved, e.g., analgesics, antihistamines, and decongestants. Under the Food, Drug and Cosmetics Act (FD&C Act), the FDA approves drugs based on "substantial evidence" of safety and effectiveness. A recommendation for approval by the committee needs to take into account the FD&C Act's evidentiary standard and the overall risk/benefit ratio for the drug in question. In the case of pleconaril, the risk/benefit consideration relates to the identification of infected patients, the variability of efficacy results, the efficacy results in smokers versus non-smokers and males versus females, and the overall safety profile of pleconaril.

Beyond the overall examination of the safety and efficacy data for pleconaril, some critical issues for you to consider as you read the Agency information and Applicant's

background materials include the study results, indication, resistance, and whether additional data would be necessary to support approval. We look forward to a very interesting meeting and thank you in advance for your time and efforts in this important meeting.

We will ask you to consider the following issues during your deliberations on this application:

- **Variable Study Results**

In two large phase 2 studies (843-020 and 843-032), pleconaril produced a median 0.0 to 0.5 day treatment effect in both the all randomized (ITT) patient population and those subjects the applicant determined to be picornavirus infected (PCR+, ITT-I). Low rates of PCR positivity, high rates of concomitant cold medication use, the inclusion of smokers, and a stringent endpoint which required all symptoms to have completely resolved may have contributed to the lack of a clearly demonstrated treatment effect in the phase 2 program.

Studies 843-043 and 843-044 were identically designed, multi-center, double-blind, randomized, placebo-controlled phase 3 studies. For the primary endpoint, the treatment effect for pleconaril was a median of 0.5 (study 843-043) and 1.5 days (study 843-044) compared to placebo. In both studies, the difference between pleconaril and placebo was statistically significant in the ITT-I population. In the ITT population, the difference reached statistical significance only in study 843-044.

Symptomatic rhinoviral infection is generally a mild, self-limited illness that typically subsides in adults in 7 to 11 days, depending on the reference cited. The median time to resolution of VRI in patients treated with pleconaril in the two pivotal trials ranged from 6.0 and 8.0 days, depending on how the analyses handled patients who did not have resolution of their VRI or who prematurely discontinued; however, the overall treatment effect was maintained.

Secondary Endpoints

Multiple secondary clinical endpoints were analyzed and included: time to patient assessment of no illness, time to reduction in symptom severity and resolution of individual symptoms, tissue use[1], time to return to baseline activity level, and the impact of concomitant cold symptom relief medication use.

FDA generally places the greatest emphasis on the primary endpoint. Secondary endpoints may be supportive but results should be interpreted with caution because of large numbers of potential comparisons, required adjustments for p values, and, in some cases, the small number of patients included in some analyses. In general, most secondary endpoints showed similar trends in favor of pleconaril as observed for the primary endpoint.

[1] Tissue use was measured during the study as a surrogate for the severity of rhinorrhea.

Patient Subgroups

Smokers versus Non-Smokers

Randomization was stratified on smoking history; 30% of study patients were classified as smokers. Compared to non-smokers, smokers experienced an overall longer duration of VRI. For smokers, time to resolution of symptoms was numerically greater in the pleconaril group compared to the placebo group.

Analysis by Gender

Gender analysis is required under FDA regulations. Analysis by gender demonstrated that the magnitude of the treatment effect among men was 0.4 days and in women it was approximately 1.0 day.

Special Populations

The studies were conducted in young, healthy individuals. Phase 1 studies suggest that no dose adjustment would likely be necessary when pleconaril is administered to elderly ($\geq$65 years) patients. In the pivotal studies, only 4% of patients were $\geq$65 years of age or older which precluded an assessment of safety or efficacy in this population. There are no data on treatment of patients with underlying cardiovascular, pulmonary disease, patients with allergic rhinitis, or patients who are immunocompromised or otherwise seriously ill. Pediatric VRI treatment trials are ongoing.

- **Identification of Picornavirus-Infected Patients and Resistance**

The applicant proposes the picornavirus-infected patients (PCR+), designated ITT-I, as the primary population for assessing efficacy. Arguably, the all randomized population (ITT) is also a valid population for assessing efficacy since pleconaril will be prescribed based on presenting symptoms without a readily available PCR assay.

Review of the NDA raised a number of questions about the methods employed to identify picornavirus-infected patients. The applicant states that 65% of patients in the two pivotal trials were infected with picornaviruses (PCR+). Infected patients were identified by an experimental TaqMan assay. TaqMan negative samples were subsequently tested for picornavirus using an experimental ELOSA system. All PCR+ samples were subsequently cultured in a rhinovirus-specific culture system; 63% of PCR+ samples were culture positive. Methodological questions include:

Primer and probe selection. The probe sequence submitted by ViroPharma contains a region of eight consecutive Gs and Cs, while the primer sequences each contain three Gs and/or Cs and on one primer these are consecutive. Tighter binding by these primers may inhibit primer dissociation and could lead to variable PCR products.

Number of cycles. The TaqMan assay is run for a total of 60 cycles. The 60-cycle reaction could allow for an increase in non-specific amplification in samples that contain weakly homologous regions. Any non-specific amplification could also have an adverse effect on the sensitivity and specificity of the assay.

Determination of threshold fluorescence level for detection of picornarvirus PCR. The threshold set by the applicant was 0.1. Based on information from the manufacturer of the assay (ViroMed), this threshold level may be too low and a more appropriate threshold might be in the range of 0.4 – 0.6. Thus, incorrect threshold placement could result in an incorrect assay definition of positivity. Also, a low threshold value could subject individual samples to assay background that could impact the relative quantitative results of the assay.

Clinical sensitivity. According to the applicant, the TaqMan assay had a sensitivity of 93% (88-97%) and specificity of 75% (72-78%). An "expanded gold standard" was used to re-evaluate sensitivity and specificity. "True positives" were considered to be either (i) virus culture positives regardless of PCR results, or (ii) TaqMan and ELOSA positives regardless of culture results. Statistically, in this analysis, it is likely that the results would favor the PCR result since one PCR assay is being "confirmed" by a second PCR assay. None of the applicant's results were independently confirmed.

Virus Culture. Two virus culture systems were utilized in the clinical studies. Only samples positive in the TaqMan assays were analyzed by cell culture, no negative specimens were tested. Positive cultures were not assessed for picornavirus positivity. Although cultures were deemed positive for plaque forming units, this was not confirmed to be due to picornavirus by pH lability, identification of the serotype, immunofluorescent assay, or DNA sequence analysis.

Additional studies to assess the performance of the TaqMan assay to identify picornavirus-infected patients are ongoing and results are expected in early March; these data will be available for discussion at the AVAC meeting. (For additional discussion about these issues, please see Appendix A).

Approximately 24% of isolates examined exhibited either a baseline lack of susceptibility (13%) or a loss of susceptibility (10.7%) to pleconaril against picornaviruses.

- **Safety**

The adult VRI safety database includes information on 2,488 patients who received at least one dose of pleconaril. Of these, 1,046 patients received the proposed regimen, 400 mg TID for 5 days (15 doses), using the proposed commercial tablet formulation. Nearly 2,000 patients received placebo in VRI studies with the tablet being of the same composition without the active ingredient.

General Adverse Events

Gastrointestinal events (abdominal pain, nausea, vomiting, and diarrhea), and headache were the most common adverse events reported in the adult VRI safety database, with headache occurring slightly more frequently among patients who received pleconaril. There were no significant laboratory abnormalities identified.

Menstrual Disorders

There was a significantly higher rate of menstrual abnormalities in women treated with pleconaril while taking estrogen/progestin-based oral contraceptives (OC) compared to women taking OCs and placebo. The frequency for pleconaril/OC users was 2.4% in 5-7 day treatment trials compared to <1% for placebo/OC users. In a six-week VRI prevention study, the frequencies were 53%, 69%, and 25% among women taking OCs with pleconaril 400 mg QD, 400 mg BID, and placebo, respectively. This will be discussed in greater detail in section V, below.

Palpitations and Tachycardia

In a theophylline/pleconaril interaction study, three of 15 subjects experienced tachycardia and palpitations when the two drugs were co-administered. In this study, subjects were naïve to theophylline prior to enrollment.

Patients in the clinical trials were otherwise healthy adults with no history of underlying cardiovascular or pulmonary disease. Palpitations and/or tachycardia with and without dizziness or syncope were reported by seven patients treated with pleconaril (0.3%) compared to two treated with placebo (0.1%). Four of the pleconaril-treated patients discontinued from studies due to these events.

II. Clinical Development Summary

The original pleconaril IND was filed June 23, 1993 by Sterling Winthrop and transferred to ViroPharma on July 5, 1995.

Pleconaril had been investigated as a treatment for enteroviral meningitis. Difficulty in identifying a correct dose, identifying patients with enteroviral meningitis with a PCR assay, determining an objective endpoint, and the inability to consistently demonstrate a treatment effect led to termination of development for this indication. Likewise, the applicant investigated pleconaril as a possible treatment for pediatric hand, foot, and mouth disease, but was not able to establish efficacy.

Pleconaril is currently under investigation for treatment of VRI in pediatric patients, prevention of VRI and treatment of enteroviral sepsis syndrome. It has been provided to patients with presumed or documented severe enteroviral diseases under a compassionate use protocol.

Development of pleconaril for treatment of acute VRI followed results of a study in which it appeared that prophylactic administration of pleconaril reduced the severity and duration of VRI in a coxsackie A-21 prevention study. Subsequently, the applicant conducted multiple phase 2 studies to assess pleconaril's activity in patients with VRI, and to prevent asthma exacerbations in patients with VRI.

The NDA contains data from six clinical trials, including two pivotal studies (843-043 and 843-044). Both pivotal studies were identical in design, and were conducted during the same time period (September-December 2000), in a similar geographic distribution (US and Canada). Both studies evaluated the safety and efficacy of pleconaril versus placebo in patients presenting with symptoms consistent with a viral upper respiratory tract infection.

Supportive studies 843-010, 843-020, and 843-032 investigated various doses (200 mg and 400 mg) and dosing schedules (BID or TID for 5 or 7 days) in patients who presented with symptoms of a VRI. Study 843-013 attempted to evaluate pleconaril's utility in preventing acute exacerbations in patients with a VRI and a history of asthma. A summary of the six trials included in the NDA is presented in Table 1.

Table 1. Adult VRI Studies

Protocol No.	Design	Regimens	No. Patients	1° Endpoint
843-010	Double-blind, randomized, placebo-controlled, parallel group in patients with Summer Flu	200 mg TID 400 mg TID Placebo 7 days	Pleconaril 200 mg: 73 400 mg: 74 Placebo: 74	Time to complete resolution of all symptoms (score of 0) for 48 hours
843-013	Double-blind, randomized, placebo-controlled, parallel group in patients with bronchial asthma	200 mg TID 400 mg TID Placebo 7 days	Pleconaril 200 mg: 87 400 mg: 84 Placebo: 83	Number and proportion of patients with an asthma exacerbation
843-020	Double-blind, randomized, placebo-controlled, parallel group in patients with picornarvirus VRI	400 mg BID 400 mg TID Placebo 7 days	Pleconaril: BID: 335 TID: 349 Placebo: 340	Time to complete resolution of the signs and symptoms of VRI (symptom score of 0) sustained for 48 hours
843-032	Double-blind, randomized, placebo-controlled, parallel group in patients with picornavirus VRI	400 mg TID Placebo 7 days	Pleconaril: 436 Placebo: 439	Time to alleviation of all VRI symptoms sustained for 24 hours
843-043 843-044	Double-blind, randomized, placebo-controlled, parallel group in patients with picornavirus VRI	400 mg TID Placebo 5 days	(843-043) Pleconaril: 526 Placebo: 526 (843-044) Pleconaril: 520 Placebo: 524	Time to resolution of rhinorrhea and alleviation (absent or mild) of other VRI symptoms without use of concomitant cold medications for 48 hours

For a review of the design and results of studies 843-010, 843-013, 843-020, and 843-032, please see Appendix B.

III. Summary of Efficacy

A. Dose and Regimen

The applicant selected 400 mg (2 x 200 mg capsules) TID for five days (15 doses) as adequate for the inhibition of picornaviral replication in the treatment of VRI. For additional information on pharmacokinetics, dose, and regimen, please see Appendix C.

B. Phase 3 Studies 843-043 and 843-044

- **Study Design and Baseline Characteristics**

Studies 843-043 and 843-044 were identically designed multi-center, double blind, randomized, placebo-controlled studies. Study 843-043 was conducted in 97 centers and 843-044 was conducted in 106 centers. Both studies were conducted in Canada and the U.S. between August and December 2000.

Patients with moderate to severe rhinorrhea and at least one other symptom of VRI (cough, nasal congestion, pharyngeal symptoms, malaise and myalgia) were randomized to receive either pleconaril 400 mg TID x 5 days or matching placebo (total 15 doses). The first dose of study medication was to be administered within 36 hours of first symptom onset (later revised to 24 hours based on post-hoc analysis of study 843-032).

Prior to the first dose, nasal mucus was collected for PCR analysis to detect picornaviral RNA using an experimental TaqMan assay system. TaqMan negative samples were re-tested using a modified experimental Enzyme-Linked Oligo Sorbant Assay (ELOSA). Only samples that were determined to be PCR+ by one of these techniques were sent for viral culture. Follow-up nasal mucus specimens for PCR were obtained on study days 3 and 6.

Patients underwent baseline assessment of VRI symptoms including rhinorrhea, nasal congestion, pharyngeal symptoms (sore throat), cough, myalgia and malaise using an ordinal numerical scoring system for severity. Rhinorrhea was scored as absent (not present, score=0), mild (1 tissue per hour, score=1), moderate (2-5 tissues per hour, score=2) or severe (more than 5 tissues per hour, score=3). Nasal congestion, pharyngeal symptoms, cough, malaise and myalgia were similarly scored as absent (not present), mild (noticeable), moderate (bothersome), or severe (interferes with activity) using the same ordinal numeric scoring system.

As shown in Table 2, both studies predominantly enrolled female Caucasians with a mean age of 36 years.

Table 2. Baseline demographic characteristics of all-randomized patients

	Study 843-043		Study 843-044	
	Pleconaril (n=526)	**Placebo (n=526)**	**Pleconaril (n=520)**	**Placebo (n=524)**
Age				
Mean (years)	36	36	36	37
Range	17-77	18-77	18-82	18-86
Gender				
Male	33%	34%	30%	28%
Female	67%	66%	70%	72%
Race				
White	75%	79%	90%	88%
Black	11%	10%	4%	5%
Hispanic	10%	7%	3%	4%
Asian	2%	2%	1%	1%
Other	2%	2%	1%	2%
Smokers	29%	29%	28%	27%
Mean (years)	12	15	12	12
Range	<1-48	<1-50	<1-60	<1-50
Pre-study cold medication use	28%	29%	32%	30%
Time between first symptom and first dose of study medication				
Mean (hours)	18.4	18.4	18	17.8
Range	3.2-26.5	2.2-25.3	1.8-39.1	2.4-43.2
Baseline symptom score[a]	9	9	9	9
Range	4-16	4-16	4-18	4-18
PCR+[b]	337 (64%)	326 (62%)	344 (66%)	356 (68%)
Viral culture + at baseline[c]	201 (38%)	196 (37%)	206 (40%)	224 (43%)

a. Maximum score=18
b. PCR + on days 1, 3 and/or 6 by research based TaqMan or modified ELOSA.
c. Viral cultures were conducted only on samples that were positive by either the TaqMan or ELOSA.

PCR+ patients had slightly higher severity scores for rhinorrhea, congestion, and sore throat compared to PCR- patients. Also, PCR+ patients were more likely to have used cold symptom relief medications prior to study entry.

- **Review of Efficacy**

Primary Efficacy Population

The applicant's primary efficacy population is all patients whose nasal mucus tested positive for picornavirus PCR during the study (infected patients, ITT-I). For completeness, analyses of efficacy in the all-randomized patient population (ITT) are also presented.

Summary of Clinical Virology

Nasal samples were tested for picornavirus using an experimental TaqMan PCR assay. Samples that tested negative by TaqMan were re-tested using an experimental Enzyme-Linked Oligo Sorbent Assay (ELOSA) in an attempt to identify additional picornavirus-infected patients. On study days 3 and 6 nasal mucus samples were collected and tested using only the TaqMan assay (instillation of 0.5 ml of saline was used to obtain nasal mucus in patients who could not expectorate mucus). Based on these procedures, the applicant concluded that 65% of patients were picornavirus PCR+ at some time during the studies.

PCR+ samples were cultured. Cultures were considered positive for rhinovirus based on presence of plaque forming units. Cultures were not further evaluated to confirm that the cultures were, in fact, rhinoviruses versus entero or other viruses.

In studies 843-043 and 043-044, 13% of baseline isolates (96/744) were not inhibited by pleconaril concentrations of 10 μg/ml. Further, during the 5-day course of treatment, reduced susceptibility to pleconaril (defined as >10-fold decrease in susceptibility compared to baseline pre-treatment isolate) emerged in 10.7% of virus isolates tested (28/263 isolates).

Primary Efficacy Endpoint and Analysis

The primary endpoint for studies 843-043 and 843-044 was the median time (days) to resolution of rhinorrhea and alleviation (absent or mild) of other symptoms of VRI without use of concomitant cold medications for 48 hours.[2]

The manner in which patients who did not reach the primary endpoint were handled impacts the analysis of efficacy. The applicant censored patients at the time immediately after their last recorded observation. The FDA analysis considered any patient who did not have resolution of their VRI or prematurely discontinued the study as treatment failures and assigned them the last possible observation as the day of healing (day 18.5). The applicant and FDA's analysis of the primary endpoint is presented in Table 3; you will note that although the treatment effect remains stable, the overall duration of illness is increased by >1 day.

[2] The endpoint evolved through numerous derivations and symptom scoring systems, and represents conclusions reached primarily from post-hoc analyses of previously completed studies. The endpoint represents assessments of four components hypothesized to be important in assessing treatment response in patients with VRI. Absence of rhinorrhea was chosen because it represents the predominant symptom of VRI. Alleviation of other symptoms to absent or mild provides outcome data on the more acute phase of VRI. The 48-hour observation period was chosen to ensure that sufficient time elapsed to document that the VRI had ended. Finally, absence of concomitant cold medication would likely ensure that patients would not report a reduced duration of VRI that could have been attributed to use of symptom relief cold medications.

Table 3. Time to resolution (days) of rhinorrhea and alleviation (absent or mild) of other symptoms of VRI without use of concomitant cold medications for 48 hours

	Study 843-043			Study 843-044		
	Placebo	Pleconaril	Diff. p-value	Placebo	Pleconaril	Diff. p-value
Applicant Analysis						
ITT (n)	526	526	0.5	524	520	0.9
N w/resolution	417 (79%)	430 (82%)	0.201	411 (78%)	423 (81%)	0.015
Median	6.9	6.4		7.1	6.2	
ITT-I (n)	326	337	0.6	356	344	1.5
N w/resolution	258 (79%)	282 (84%)	0.037	286 (80%)	290 (84%)	0.001
Median	7.2	6.6		7.7	6.2	
FDA Analysis						
ITT (n)	526	526	0.5	524	520	1.0
Median	7.5	7.0	0.13	80	7.0	0.014
ITT-I (n)	324	334	0.5	350	343	1.5
Median	7.5	7.0	0.03	8.5	7.0	0.004

Efficacy Results According to Randomization Strata

Randomization was stratified based on smoking status and pre-treatment use of symptomatic cold relief medications.

Smokers Compared to Non-Smokers

Approximately 30% of study participants had a smoking history. At baseline, smokers reported slightly more severe cough, otherwise there were no significant demographic differences between smokers and non-smokers. A history of smoking clearly impacted the duration of VRI overall, and resulted in numerically negative treatment effect for pleconaril. The FDA analysis of the primary endpoint based on smoking status is presented in Table 4.

Table 4. Time (days) to the primary endpoint in smokers and non-smokers

	Study 843-043		Study 843-044	
	Placebo	Pleconaril	Placebo	Pleconaril
ITT Smokers (n)	152	153	138	136
Median	8.0	7.5	7.5	8.5
ITT Non-smokers (n)	374	374	386	384
Median	7.5	6.5	8.0	6.0
ITT-I Smokers (n)	76	102	90	87
Median	8.0	9.0	8.5	9.0
ITT-I Non-smokers (n)	224	219	249	240
Median	7.5	6.5	8.5	6.5

Also, smokers treated with pleconaril experienced more acute complications of VRI (otitis media, bronchitis, sinusitis, and pneumonitis) compared to non-smokers.

Pre-Treatment Cold Medication Users Compared to Non-Users

At study entry, patients were stratified on pre-treatment use of cold symptom relief medications. Approximately 30% of participants in both studies used at least one dose of symptom relief medication (e.g., acetaminophen, ibuprofen, dextromethorphan, pseudoephedrine, Dristan, Dimetapp, echinacea, zinc, or NyQuill,) prior to initiation of study medication. Patients with moderate/severe pharyngeal symptoms, myalgia, and malaise appeared to be more likely to have used pre-treatment cold relief medications. The analysis of the primary endpoint by use and non-use of pretreatment cold relief medications is presented in Table 5.

Table 5. Time (days) to primary endpoint in users and non-users of pre-treatment cold medications

	Study 843-043		Study 843-044	
	Placebo	**Pleconaril**	**Placebo**	**Pleconaril**
ITT users (n)	138	138	163	162
Median	8.0	8.5	8.0	8.5
ITT non-users (n)	388	388	361	358
Median	7.0	6.5	8.0	6.5
ITT-I users (n)	85	101	119	120
Median	9.5	8.5	9.0	8.5
ITT-I non-users (n)	215	220	237	224
Median	7.5	6.5	7.4	6.0

Efficacy Analysis by Gender

Analysis of the primary endpoint by gender demonstrated:

- In men, there was a median 0.4 day treatment effect in favor of pleconaril over placebo, 5.7 versus 6.1 days (p=0.210).
- Women treated with pleconaril experienced a median 1.0-day faster time to resolution compared to women treated with placebo, 7.0 versus 8.0 days.

Analysis of Secondary Endpoints

Multiple secondary clinical endpoints were analyzed and included: time to patient assessment of no illness, time to reduction in symptom severity and resolution of individual symptoms, tissue use, time to return to baseline activity level, and the impact of concomitant cold symptom relief medication use.

FDA generally places the greatest emphasis on the primary endpoint. Secondary endpoints may be supportive but results must be interpreted with caution because of the large number of potential comparisons, required adjustments for p values, and, in some cases, the small number of patients included in the analysis. The results of a number of these analyses are presented for completeness.

Analyses of time to patient assessment of no cold are presented in Table 6.

Table 6. Time (days) to patient assessment of "no cold"

	Study 843-043		Study 843-044	
	Placebo	**Pleconaril**	**Placebo**	**Pleconaril**
ITT (n)	526	526	524	520
Median	6.7	6.0	6.9	5.9
ITT-I (n)	326	337	356	344
Median	6.9	6.5	7.4	6.3

Analysis of time to resolution of the individual symptoms of VRI was assessed and the results are presented in Tables 7a and 7b.

Table 7a. Median time (days) to resolution (score=0) of symptoms of VRI, ITT

	Study 843-043.		Study 843-044	
	Placebo	**Pleconaril**	**Placebo**	**Pleconaril**
Rhinorrhea (n)	490	486	495	489
	6.0	6.5	6.5	5.5
Congestion (n)	467	472	482	471
	60	6.0	6.0	6.0
Cough (n)	411	387	402	399
	6.0	6.0	7.0	5.5
Pharyngitis (n)	396	352	375	380
	4.0	3.5	3.5	3.0
Malaise (n)	448	448	454	445
	4.5	4.0	4.0	4.0
Myalgia (n)	366	328	337	344
	4.0	3.0	4.0	3.5

Table 7b. Median time (days) to resolution (score=0) of symptoms of VRI, ITT-I

	Study 843-043.		Study 843-044	
	Placebo	**Pleconaril**	**Placebo**	**Pleconaril**
Rhinorrhea (n)	284	302	323	311
	7.0	6.0	7.5	6.0
Congestion (n)	274	294	318	305
	6.5	5.5	6.5	6.0
Cough (n)	242	238	262	258
	6.5	6.5	7.0	6.0
Pharyngitis (n)	228	212	243	234
	3.5	3.0	3.5	3.0
Malaise (n)	261	276	307	287
	4.5	4.0	4.0	3.5
Myalgia (n)	206	192	223	222
	3.5	3.0	3.5	3.0

Analysis of nights disturbed by VRI symptoms is presented in Table 8.

Table 8. Median nights with sleep disturbance due to VRI symptoms

	Study 843-043		Study 843-044	
	Placebo	**Pleconaril**	**Placebo**	**Pleconaril**
ITT w/sleep disturbance (n)	440	414	427	419
Median	3.0	2.0	3.0	2.0
ITT-I w/sleep disturbance (n)	282	276	300	289
Median	3.0	2.0	3.0	2.0

There was no difference in time to return to baseline level of activity between pleconaril and placebo for the ITT or ITT-I populations, median 3.0 days.

At baseline each patient was dispensed acetaminophen and dextromethorphan and instructed to use these relief medications only if necessary. All use was to be documented in the patient diaries. Approximately 50% of study participants used symptomatic relief medications during the study, and there were no differences in the frequency or duration of use between pleconaril and placebo-treated patients.

V. Safety Summary

The adult VRI safety database includes information on 2,488 patients who received at least one dose of pleconaril; 1,046 of whom received the proposed dose of 400 mg TID for 5 days (15 doses), using the proposed commercial tablet formulation.

Gastrointestinal events (abdominal pain, nausea, vomiting, diarrhea), and headache were the most common adverse events reported in the adult VRI safety database. Of significant concern is the apparent higher rate of menstrual disorders among females treated with pleconaril and concomitant progestin and/or estrogen-based oral contraceptives (OCs). Another potential concern was the occurrence of tachycardia and palpitations that was temporally related to administration of pleconaril alone and in combination with theophylline. There were no significant changes in clinical laboratory or hematologic parameters in the phase 2 and 3 VRI trials reviewed where pleconaril was dosed for 5-7 days.

Treatment emergent adverse events (all cause and all severity) observed in the two pivotal VRI studies are presented in Table 9.

Table 9. Treatment-emergent adverse events in pivotal adult VRI studies, (%)

Event	Pleconaril (n=1046)	Placebo (n=1040)
Headache	23	21
Diarrhea	7	7
Nausea	6	4
Vomiting	2	2
Abdominal pain	2	4
Sinusitis	3	2
Bronchitis	3	3
Increased cough	3	3
Pain	3	2
Fever	1	2
Dizziness	2	1
Hematuria	1	<1
Otitis media	1	<1
Rhinitis	2	3
Allergic reactions	<1	<1
Rash	<1	<1
Menstrual disorders	2	1
Abnormal LFTs	<1	1
Palpitations/tachycardia	<1	<1

There were no significant differences in the frequency of adverse events between PCR+ and PCR- patients in studies 843-043 and 843-044.

Headache, primarily mild in severity, has been reported consistently by 18-20% of patients in the VRI safety database. In the two pivotal studies, the frequency of headache was similar as was the number of patients who discontinued due to headache, four pleconaril and six placebo. Although some cases of headache were quite likely due to the underlying VRI, many were reported during treatment and therefore may have been treatment related. Other etiologies for headaches have not been suggested.

Gastrointestinal-related adverse events were reported at a similar frequency across the studies in the VRI safety database as shown in Table 9. In the two pivotal studies, 17 (1.6%) and six (.05%) pleconaril and placebo-treated patients, respectively, discontinued due to GI adverse events. Review of discontinuations due to GI events demonstrated a pattern: onset of symptoms was within 1-2 days of initiation of study medication with resolution within 1-2 days of discontinuation. In general, gastrointestinal symptoms are not part of the typical constellation of symptoms reported by patients with VRI. One possibility is that the GI events are formulation related. The placebo and pleconaril formulations used in the pivotal studies contain sodium laurel sulfate (SLS).[3] Other etiologies for these gastrointestinal events have not been suggested.

[3] SLS is a generally recognized as safe (GRAS) anionic emulsifying, detergent used as a tablet lubricant and wetting agent in cosmetics and topical pharmaceutical products. It is a moderately toxic agent with irritating effects to the eyes, skin, mucous membranes, upper respiratory tract, and stomach. Handbook of Pharmaceutical Excipients, 3rd edition, Kibbe AH, ed; 2000, 487-89.

Menstrual Disorders

There was a significant increase in menstrual disorders (such as epimenorrhea, intermenstrual bleeding, menstrual disorder, amenorrhea, menorrhagia, early menses, prolonged menstrual cycle, and delayed menses) among women treated with pleconaril and using progestin/estrogen-containing OCs.

Menstrual disorders were identified in 7/229 (3.1%) of women who were taking OCs during 5-7 days of treatment with pleconaril compared to 0/223 who were treated with placebo. Among women who received pleconaril but were not using OCs, the rate of menstrual disorders was 0.1%.

Additional information on menstrual disorders from a recently completed six-week prophylaxis study were submitted and reviewed. The observed results are suggestive of a dose response relationship. At least one of the menstrual disorders listed above was observed in 20 (25%), 52 (53%), and 40 (69%) women taking placebo, pleconaril 400 mg QD, or pleconaril 400 mg BID and an OC. The most common events were epimenorrhea and intermenstrual bleeding.

During week one of the prophylaxis study, the prevalence of menstrual disorders was 3%, which was the same as the prevalence in the 5-7 day treatment trials. The prevalence peaked by week three to 28%, and began to decrease by week seven.

Also, there appeared to be a potential dose response for the occurrence of epimenorrhea and intermenstrual bleeding among women receiving pleconaril and not taking OCs; however the numbers of patients in this analysis are very small.

In animal studies, pleconaril was not found to be mutagenic, teratogenic, or genotoxic, and did not negatively impact viability or normal development, including reproductive performance of the offspring. In a 6-month toxicology study conducted in rats, mild to moderate macroscopic uterine horn distention was observed at necropsy in low (10 mg/kg BID) and high dose animals (50 mg/kg BID). Corresponding microscopic uterine horn dilatation was observed in two of two low dose animals and five of 15 high dose animals. These findings were also present in one high dose animal at the end of the two-month recovery period. At the time the toxicology study was reviewed, these findings were not considered sufficient to warrant a hold for a dosing duration of five days, since no similar toxicities were noted in one-month dog and rat studies or in six month dog studies.

A total of 13 (eight pleconaril and five placebo) pregnancies have been reported in women receiving pleconaril in 5-7 day treatment and six-week prevention studies. Of the eight pregnancies that occurred in pleconaril recipients, two occurred in women taking an OC at the time they became pregnant. One pregnancy terminated in an abortion and one pregnancy is ongoing. All the other women were using barrier methods at the time they became pregnant.
The etiology of the menstrual disorders has not yet been determined. Possible mechanisms include CYP 3A4 induction, changes in intestinal flora, interference in

enterohepatic circulation, displacement of albumin binding of estrogen, changes in sex hormone binding globulin, changes in steroid receptor binding, and partial antagonism of estrogen receptors.

Two studies investigating changes in serum progesterone, luteinizing hormone and follicle-stimulating hormone are ongoing. A third study investigating the possibility of induction of the CYP 3A4 enzyme system using midazolam is also ongoing. Very preliminary data suggests that pleconaril causes induction of CYP 3A4 and decreases ethinyl estradiol exposures. Additional data from these studies will be presented at the AVAC meeting.

Tachycardia/Palpitations

A potential pharmacodynamic interaction between pleconaril and theophylline was identified. During the conduct of an interaction study, four of 14 subjects experienced palpitations, tachycardia, dizziness, and/or syncope, during the combination dosing period. In addition, general adverse events of palpitations, nausea, vomiting, abdominal pain, and dizziness were reported more frequently during the theophylline/pleconaril periods. At the time of these events, no significant changes in either theophylline or pleconaril pharmacokinetics noted were noted.

In the adult VRI database 18 patients (eight placebo and 10 pleconaril) reported cardiac-related adverse events for which a cardiac etiology could not be ruled out. Nine patients, seven pleconaril and two placebo, complained of tachycardia and/or palpitations. Of these, six of the pleconaril patients reported tachycardia and/or palpitations within one hour of ingestion of pleconaril, four discontinued from treatment due to tachycardia and/or palpitations, one patient's event was considered serious. No patients underwent re-challenge following resolution of the events.

There were no apparent differences in heart rate or blood pressure noted in the VRI safety database.

VI. Proposed Phase 4 Studies

The applicant has proposed the following post-approval activities:

- Conduct additional pediatric VRI studies using an endpoint of time to 50% reduction in symptoms
- Assess pleconaril's ability to prevent VRI in patients with underlying pulmonary disease (e.g., chronic asthma and COPD)
- Assess transmission of VRI and development of resistance

VII. Issues for Discussion

Listed below are a number of issues for you to consider during the discussion period. Please note that these issues may change prior to the meeting.

1. Has the applicant established efficacy of pleconaril for treatment of acute picornavirus VRI in adults?

Please consider the following points as you discuss this question:

- Methods used to identify infected patients
- Variability of treatment effect across the phase 2 studies, and studies 843-043 and 843-044
- Determination of the primary efficacy population
- The need to institute treatment within 24 hours of onset of the first symptoms
- Results in males versus females
- Results in smokers versus non-smokers

1b. If the answer to question 1 is yes, please provide your recommendations for labeling. Please include a discussion of the populations and sub-populations for which pleconaril should or should not be labeled, and any recommendations for statements to clarify how pleconaril should be used.

1c. If the answer to question 1 is no, please discuss what additional data would be helpful to establish pleconaril's efficacy.

2.Has the applicant demonstrated safety in adult patients with symptoms of acute VRI?

Please consider the following points as you discuss this question:

- The types and frequencies of adverse events are generally similar between pleconaril and placebo, with headache and gastrointestinal symptoms occurring most frequently.
- The frequency of menstrual disorders occurring in females using OCs while being treated with pleconaril.
- Tachycardia and palpitations were reported in <1% of otherwise healthy patients treated with pleconaril, and were pronounced in a theophylline/pleconaril interaction study. Patients with significant pulmonary and cardiovascular disease were not included in any of the studies.

2a. If the answer to question 2 is yes, please provide any recommendations for labeling. Please include a discussion of the populations and sub-populations for which pleconaril should or should not be labeled, and recommendations for specific safety related statements that should be included in the labeling.

2b. If the answer to question 2 is no, please discuss what additional data would be helpful to establish pleconaril's safety.

3.Please provide comments on the applicant's proposed phase 4 studies. Please also provide suggestions for any other study designs or patient populations that should be studied as phase 4 commitments.

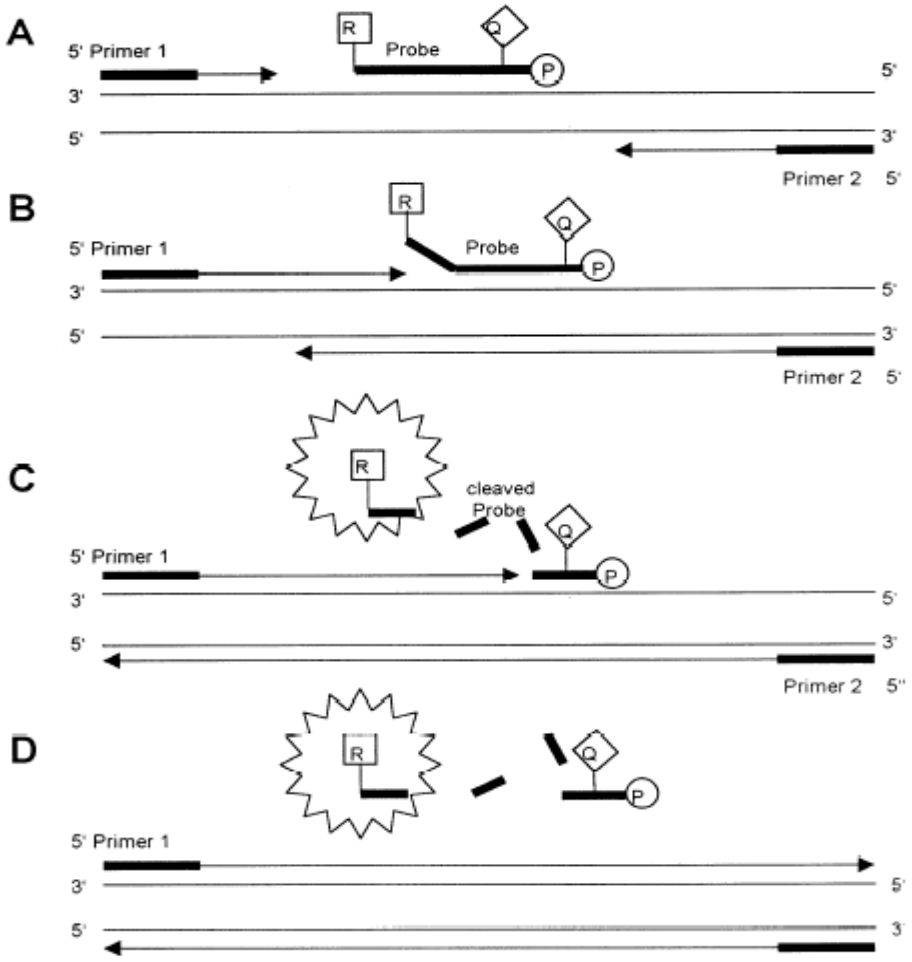
APPENDIX A
SUMMARY OF VIROLOGIC METHODS

Additional studies to assess the performance of the TaqMan assay to identify picornavirus-infected patients are ongoing and results are expected in early March; these data will be available for discussion at the AVAC meeting and may supercede the concerns outlined below.

General Overview of the Assay

The basis for Taqman PCR is to continuously measure PCR product accumulation using a dual-labeled flourogenic oligonucleotide probe called a TaqMan® probe. This probe is composed of a short (ca. 20-25 bases) oligodeoxynucleotide labeled with two different fluorescent dyes. On the 5' terminus is a reporter dye and on the 3' terminus is a quenching dye. This oligonucleotide probe sequence is homologous to an internal target sequence present in the PCR amplicon. When the probe is intact, energy transfer occurs between the two flourophors and the quencher quenches emissions from the reporter. During the extension phase of PCR, the probe is cleaved by 5' nuclease activity of Taq polymerase thereby releasing the reporter from the oligonucleotide-quencher and producing an increase in reporter emission intensity

The fluorescence intensity of reporter and quencher dyes is measured and the increase in normalized reporter emission intensity over the course of the amplification is calculated. The results are then plotted versus time, represented by cycle number, to produce a continuous measure of PCR amplification. To provide precise quantification of initial target in each PCR reaction, the amplification plot is examined at a point during the exponential phase of amplification. This is accomplished by assigning a fluorescence threshold above background and determining the time point at which each sample's amplification plot reaches the threshold (defined as the threshold cycle number or CT). Differences in threshold cycle number can be used to quantify the relative amount of PCR target contained within each tube.



Critical Assay Parameters

TaqMan® Probe and Primers

1) As noted by Applied Biosystems, the manufacturer of the TaqMan system, the use of Primer Express® software in designing primers and probes is critical for the successful development of an assay. The assay under review did use this software for primer and probe design, however there are at least two areas of concern. As noted by the Primer Express software:

TaqMan® Probe:

- Avoid runs of an identical nucleotide. This is especially true for guanine (G), where runs of four or more Gs should be avoided

TaqMan® Primers:

- The five nucleotides at the 3' end should have no more than two G and/or C bases

2) Because of heterogeneity between rhino and enteroviruses, and that the assay used only two primers, it is not clear how effective the assay can be at detecting all various serotypes. In addition, as noted below there was significant variation in the level of detection of the serotypes. Therefore, these primer-binding characteristics have the ability to significantly affect both the sensitivity and specificity of the assay.

Threshold Value

The threshold is the numerical value assigned for each run that reflects the average DRn (change in fluorescence) during the initial cycles of PCR (baseline). The threshold should be placed in the region of exponential amplification across all of the amplification plots. This region is depicted in the log view of the amplification plots as the portion of the plot that is linear. The threshold line should neither be placed in the plateau phase or in the initial linear phase of amplification. In the case of the ABI 5700 the threshold is manually adjusted so there may be variation in where individual investigators believe placement should be set.

It is possible that the threshold currently set for this assay, 0.1, may be too low and a more appropriate threshold would be in the range of 0.4 – 0.6.1. While there is a range of threshold values that could be appropriate, incorrect threshold placement could result in an incorrect assay definition of positivity.

A low threshold value would subject individual samples to assay background and could impact the relative quantitative results of the assay.

In the clinical studies, 67% (6/9) of the assay failures were due to the negative control crossing the threshold value.

Number of Cycles

The current assay is run for a total of 60 cycles. In general, most assays are run for a maximum of 45 cycles.

The 60-cycle reaction could allow for an increase in non-specific amplification in samples that contain weakly homologous regions.

Any non-specific amplification would also have an adverse effect on the sensitivity and specificity of the assay.

Possible resolutions to this issue would include analyzing the reaction products by ethidium bromide-stained agarose (acrylamide) gel electrophoresis, Southern blotting/probe hybridization, or fluorescence assay. These were requested of ViroPharma but have not been received to date.

Pre-Clinical Studies

Validation Reports

1) The validation report in the original NDA submission identified the primers and probe selected for the assay using the appropriate Primer Express software and these would generate a 67-68 nucleotide (nt) fragment.

A validation report from ViroMed, who performed the TaqMan assays for the applicant, states that the primers and probe selected using the Primer Express software generated a 150 nt fragment; ViroPharma will clarify the exact length of the nt fragment.

Detection Limits (Analytical Sensitivity)

1) Detection limits vary widely between serotypes. Detection limits were expressed in genome equivalents (GE)/plaque forming units (pfu). Five human rhinovirus (HRV) serotypes representing both major (serotypes 14,16, and 89) and minor (serotypes 1A and 1B) receptor binding groups were used to establish the limit of detection.

	Analytical Sensitivity	
HRV	GE (RNA) per reaction	GE/pfu
1A	30	132
1B	50	1055
14	1289	12675
16	47	408
89	2036	33,068

Given that particle/pfu ratios for picornaviruses are generally between 200 –1000[2] and since there should be no more than one genome per virus particle (or less), these GE/pfu ratios appear to be overstated.

The analytical sensitivity varied over 60-fold, from 30 to 2,036 GE per reaction depending on virus serotype.

There is a discrepancy between the claimed limit of detection (LOD) of the TaqMan assay. In the ViroMed validation report the LOD is stated as 1.0 pfu/ml while in the studies submitted by ViroPharma, the listed LOD is 10 pfu/ml.

The exact quantitative nature of the TaqMan assay cannot be evaluated as the calibrators also serve as the positive control for the assay and therefore will only control for substantial reagent failure. When control and calibrators are the same material, the calibrators will appear to function within the parameters of each assay but there may be substantial inter-assay variation that will not be detected.

When used with spiked clinical material (nasal mucus) the detection limits of the TaqMan assay varied among the serotypes by 2000 fold.

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Clinical Data

Virus Culture

1) Two virus culture systems were utilized in the clinical studies.
   a) In one, samples of nasal mucus specimens were inoculated into WI-38 cells (human embryonic lung cells), inculbated at 33° C, and examined for cytopathic effect (cpe) over the course of 14 days.
   b) The second culture system employed Hela I-CAM cells with other conditions being similar.
2) Only samples positive in the TaqMan assay were analyzed by cell culture, no negative specimens were tested.
3) Positive cultures were not assessed for picornavirus positivity. Although cultures were deemed positive for cpe, this was not confirmed to be due to picornavirus by pH lability, identification of the serotypes, immunofluorescent assay, or DNA sequence analysis.

Thus, while a positive culture was suggestive of picornavirus, the applicant provided no evidence for either the presence or absence of actual picornavirus infection.

Clinical Sensitivity

1) In Study 843-032 when compared to culture, the TaqMan assay had a sensitivity of 93% (88-97%) and specificity of 75% (72-78%).
2) An "expanded gold standard" was used to re-evaluate sensitivity and specificity. "True positives" were considered to be either (i) virus culture positives regardless of PCR results, or (ii) TaqMan and ELOSA positives regardless of culture results.

There is no valid measure for specificity for either assay, and neither result has been independently confirmed.

Statistically this analysis will always favor the PCR result, since one PCR assay is being "confirmed" by a second PCR assay.

APPENDIX B
REVIEW OF SUPPORTIVE STUDIES

The design and primary endpoint analysis for each of four phase 2 studies are presented below. Presented below are analyses of the primary endpoint for each study.

Study 843-010 was the first study to investigate the safety and efficacy of pleconaril in the treatment of VRI. The primary endpoint was time to complete resolution of all systemic symptoms (score of 0) for 48 hours among the all randomized (ITT) patient population. Nasal mucus was PCR+ for picornavirus in 29% percent of patients, too few upon which to conduct an analysis.

Table 2. Time (days) to complete resolution of all systemic symptoms for 48 hours

	Placebo	Pleconaril 200 mg TID	Pleconaril 400 mg TID
ITT (n)	74	73	74
Median	10	9	10
p-value versus placebo		0.501	0.504

Study 843-013 evaluated two doses of pleconaril (200 mg or 400 mg TID for seven days) in the treatment of rhinovirus VRI in patients with bronchial asthma. The purpose was to determine if treatment of VRI in patients with asthma might reduce the morbidity and mortality associated with such exacerbations. The low rates of asthma exacerbations and picornavirus PCR+ patients precluded any efficacy assessments.

Study 843-020 evaluated the safety and efficacy of two doses of pleconaril (400 mg BID or TID for seven days) for treatment of picornavirus VRI. The primary endpoint was time to reduction of a composite symptom score to 0 (all signs and symptoms absent) for two consecutive days (48-hours) in adult patients with acute picornavirus VRI. Nasal mucus was PCR+ for picornavirus in 41% of patients.

Table 3. Time (days) to resolution of all VRI symptoms sustained for 48 hours

	Combined Placebo	Pleconaril 400mg BID	Pleconaril 400mg TID
ITT (n)	340	335	347
Median	8	8	8
p-value versus placebo		0.31	0.011
ITT-I (n)	128	136	147
Median	8	8	9
p-value versus placebo		0.69	0.70

Study 843-032 evaluated the safety and efficacy of pleconaril 400 mg TID or matching placebo TID for seven days (total 21 doses) in patients >14 years of age presenting with acute VRI. The first dose of study medication was to be administered within 36 hours of the onset of symptoms. The primary endpoint was time to alleviation of all VRI symptoms sustained for 24 hours. Nasal mucus was PCR+ for picornavirus in 43% of patients.

Table 4. Time (days) to resolution of all VRI symptoms sustained for 24 hours

	Placebo	**Pleconaril 400mg TID**	**Difference p-value**
ITT (n)	439	436	0.0
Median	9.0	9.0	0.7
ITT-I (n)	280	240	0.5
Median	9.5	9.0	0.11

APPENDIX C
SUMMARY OF PHARMACOKINETICS, DOSE AND REGIMEN

The applicant conducted an extensive PK development program. Unless otherwise noted, the PK studies involved healthy volunteers between 18 and 75 years of age. Relevant findings and issues raised by pharmacokinetic studies that are applicable to the adult VRI indication include:

- Pleconaril reaches maximal plasma concentration (C_{max}) about 3.0 hours (range 2.0-5.0 hours) after oral administration.
- The bioavailability of pleconaril is increased by 4-6.5 fold after administration of pleconaril with a meal high in calories, fat, and protein, as compared to administration of pleconaril in a fasted state.
- Pleconaril is highly bound (99.8%) to plasma proteins.
- Following oral absorption, pleconaril displays a bi-exponential disposition profile with a short alpha half-life (2-3 hours) and a long terminal half-life (180 hours).
- The pharmacokinetics following a single dose may not predict multiple dose pharmacokinetics, due to the prolonged half-life after multiple doses (1010 hours after 400 mg TID for 5 days) compared to that after single dose (180 hours).
- Plasma accumulation of pleconaril is modest (2-fold) after pleconaril 400 mg TID for 5 days. Steady state is not reached after 5 days of treatment with pleconaril.
- Mass balance was not achieved following 288 hours of collection of urine and feces after a single radiolabeled oral dose of 200 mg pleconaril under fed conditions (37.6% of administered radioactivity recovered in urine and feces).
- Pleconaril is excreted in urine and feces as multiple inactive metabolites. The cytochrome P450 drug metabolizing enzymes are not significantly involved in the major biotransformation pathways of pleconaril. Therefore, co-administered drugs that are CYP450 substrates, inhibitors or inducers are not expected to alter the pharmacokinetic profile of pleconaril.
- *In vitro* studies showed that pleconaril may be a weak CYP1A2 (Ki: 8.8 μM), CYP2C9 (Ki: 6.4 μM), and CYP2C19 (Ki: 10 μM) inhibitor. The *in vivo* pleconaril-theophylline drug-drug interaction study showed that pleconaril increased theophylline AUC by 15%, but not Cmax. Palpitations, syncope and tachycardia were observed during the theophylline and pleconaril combination treatment but not during theophylline alone treatment. These symptoms appeared to be unrelated to theophylline AUC or Cmax. Pleconaril had no effect on pharmacokinetics of S- or R-warfarin.
- An increased incidence of menstrual disorders, including break-through bleeding, was observed in pleconaril treated women who took oral contraceptives, both in prophylaxis and treatment studies. The potential for pleconaril induction of CYP450 enzymes is currently under investigation.
- The pharmacokinetic profile of pleconaril is not clinically significantly changed due to age or renal impairment.

- Hepatic impaired subjects have 40% lower pleconaril exposures compared to healthy subjects after a single 400 mg pleconaril oral liquid dose. The difference may be due to lower fat and meal consumption in hepatic impaired subjects compared to healthy subjects during the study or reduced albumin level in hepatic impaired subjects. Thus, the results of this study are inconclusive.
- The population PK study was inconclusive due to insufficient blood samples, inappropriately fixed parameters (using ka, Q/F, V_2/F and t_{lag} values from Phase I studies with different formulations of pleconaril), and poor prediction of observed concentrations.
- The dose selected (400 mg TID for 5 days) may not be optimal based on the free drug concentrations achieved in the plasma and estimated nasal tissue concentration from a rat model, compared to non-protein adjusted MIC_{90} of pleconaril for piconavirus. Also, no higher dose was studied and no exposure-response was evaluated.