

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
21-785

MICROBIOLOGY REVIEW

Microbiology Review
Division of Antiviral Drug Products (HFD-530)

NDA#: 21-785	Serial #: 000	Reviewer:	N. Battula
Date submitted:	June 17, 2004	Date received:	June 18, 2004
Date assigned:	July 01, 2004	Date reviewed:	December 6, 2004

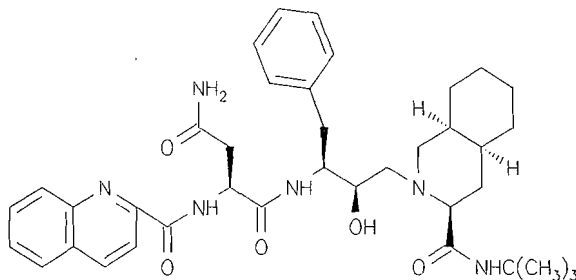
Additional submissions reviewed

	<u>Supplement #</u>	<u>Date of Correspondence</u>	<u>Date of Receipt</u>
NDA: 21-785	N-000	September 24, 2004	September 27, 2004
NDA: 21-785	N-000	November 2, 2004	November 3, 2004
NDA:21-785	N-000	December 7, 2004	December 15, 2005

Sponsor: Hoffmann-La Roche Inc.
340 Kingsland Street
Nutley, NJ 07110-1199

Product name(s): Invirase®
Non-proprietary: Saquinavir mesylate
Chemical: N-tert-butyl-decahydro-2-[2(R)-hydroxy-4-phenyl-3(S)-[[N-(2-quinolylcarbonyl)-L-asparaginy]amino]butyl]-(4aS,8aS)-isoquinoline-3(S)-carboxamide

Structural formula:



Molecular formula = $C_{38}H_{50}N_6O_5$ Molecular weight = 671

Dosage form/route of administration: Tablets/ Oral

Indication: Treatment of HIV-1 infection in combination with other antiretroviral agents

Related documents: NDAs: 20-628 and 20-828 and IND 41,099

Background and Summary: Hoffmann-La Roche Inc. submitted this NDA for Invirase® in support of a new formulation, Invirase 500 mg film coated tablets (Invirase FCT). The application is based on data from bioavailability and bioequivalence studies, and, the chemistry manufacturing controls information. The submission also includes a supplemental safety and efficacy data from two phase 4 clinical studies, MaxCmin-1 and MaxCmin-2. FDA granted priority review for this application in consideration of substantial reduction in pill burden and potential benefits to the HIV-1 infected patients.

The submission has no new microbiology studies with the Invirase 500 mg FCT development program. However, following the approval of the NDA 20-828/SE2-015 for Fortovase (saquinavir), and NDA 20-628/SE2-020 for Invirase (saquinavir mesylate) on December 24, 2003, a post approval commitment plan was submitted to the sponsor. As apart of the microbiology portion of the phase 4 commitments the sponsor was requested to: (1) Submit phenotypic and genotypic data from the MaxCmin-1 and MaxCmin-2 studies. The data should include phenotypic susceptibility and HIV-1 genotypes at baseline and sequential changes during treatment with ritonavir-boosted saquinavir and (2) Submit data on the in vitro combination effects (e.g. additive, synergistic or antagonistic) effects of saquinavir with all approved antiretroviral agents. In addition, FDA also requested that Roche conduct studies on the in vitro antiviral activity of saquinavir against different clades of HIV-1 and the antiviral activity of saquinavir against HIV-2 isolates.

Subsequent to the initial submission of the NDA application, Roche provided in vitro studies on the antiviral activity of saquinavir against different clades of HIV-1, in vitro antiviral activity of saquinavir against HIV-2 isolates, in vitro combination activity studies of saquinavir with all of the currently approved antiretroviral agents, and partial data sets on the genotypic changes in patient samples from the MaxCmin-1 and MaxCmin-2 studies. These microbiology studies are evaluated in this review.

In vitro antiviral activity of saquinavir against different clades of HIV-1: Studies on the determination of the in vitro antiviral activity of saquinavir against different clades of HIV-1 were determined the antiviral activity of saquinavir against multiple isolates from each of the different HIV-1 clades from samples that were randomly selected from their library of HIV-1 isolates. The results presented in Table 1 show that saquinavir IC₅₀ (50% inhibitory concentration) values for HIV-1 clades A to H ranged from 0.9 to 2.5 nM with the geometric mean of non-B clade isolates similar to that of clade B isolates. Therefore, saquinavir would be expected to show activity in vivo against the various HIV-1 clades.

This additional information on the antiviral activity of saquinavir was added to the microbiology section of the package insert.

Table 1. Antiviral activity in vitro of saquinavir against some HIV-1 clades

HIV-1	N	Geometric mean IC ₅₀ (nM)	IC ₅₀ Range (nM)
Clade A	5	0.989	0.90-1.30
Clade AE	5	1.318	1.20-1.40
Clade C	5	1.357	1.00-1.60
Clade D	5	1.441	1.00-1.90
Clade F	5	1.726	1.50-2.20
Clade G	5	1.527	1.10-2.30
Clade H	2	2.000	1.60-2.50
All Non-B clades	32	1.406	0.90-2.50
Clade B	5	1.381	1.10-1.70

In vitro antiviral activity of saquinavir against HIV-2 isolates: The studies on the in vitro antiviral activity of saquinavir against HIV-2 isolates were done by _____ company, _____. In these studies phytohemagglutinin-stimulated human peripheral blood mononuclear cell cultures were infected with different isolates of HIV-2 at a multiplicity of infection of 0.1. The extent of virus replication in the presence of graded concentrations of saquinavir was determined by measuring the reverse transcriptase activity in the virus infected cell supernatants. The reverse transcriptase activity was quantified by the incorporation of [³H]-TMP into _____ template. Inhibition of virus replication was expressed as IC₅₀ values. Cytotoxicity of saquinavir was assessed by the tetrazolium dye XTT assay and expressed as TC₅₀ (50% reduction in cell viability).

The data presented in Table 2 show that the 50% and 90% inhibitory concentrations of saquinavir against different HIV-2 isolates ranged from 0.25 nM to 14.6 nM and 4.65 nM to 28.6 nM, respectively. The outlier IC₉₀ value for HIV-2 CDC-310340 showing >1000 nM was not considered in the IC₉₀ value calculation because of the overall inhibitory concentrations with the 7 different HIV-2 isolates and the IC₅₀ value for HIV-2 CDC-310340 isolate (0.53 nM). The activity in vivo against HIV-2 infected individuals is expected to be variable. The therapeutic index (TC₅₀/IC₅₀) in these studies ranged from >68.7 to >3977. This additional information on the antiviral activity of saquinavir against different HIV-2 isolates was added to the microbiology section of the package insert.

Table 2. Antiviral activity in vitro of saquinavir against HIV-2 isolates

HIV-2 isolate	IC ₅₀ (nM)	IC ₉₀ (nM)	TC ₅₀ (nM)	Therapeutic Index
HIV-2 _{9924A}	14.6	28.6	>1000	>68.7
HIV-2 _{60415K}	0.26	10.3	>1000	>3824
HIV-2 _{CDC-77618}	1.83	9.74	>1000	>548
HIV-2 _{CDC-310340}	0.53	>1000	>1000	>1880
HIV-2 _{MVP-15132}	0.25	4.65	>1000	>3957
HIV-2 _{CDC-310319}	2.43	7.85	>1000	>411
HIV-2 _{CBL-20/H9}	7.0	37.2	>1000	>143

In vitro combination activity studies of saquinavir with approved antiretroviral agents:

The studies on the in vitro combination antiviral activity of saquinavir with all of the currently approved antiretroviral agents were done by a

_____ . The anti-HIV-1 activity of saquinavir in combination with each of the currently approved 18 antiretroviral agents was carried out in a two drug combination (saquinavir + test drug) effect format. Analysis of the combination effect was performed using the _____ three-dimensional model. The combination assays were conducted in CEM-SS T-lymphocytes infected with HIV-1_{IIIB} in a microtiter plate format. Antiviral activity was evaluated by measuring the virus induced cytopathic effect on CEM-SS T-lymphocytes.

The combination anti-HIV-1 activity was expressed in effect volume units such as additive volume, synergy volume or antagonistic volume. Saquinavir in combination with each of the 18 currently approved drugs (8 nucleoside analogue reverse transcriptase inhibitors, 3 non nucleoside analogue reverse transcriptase inhibitors, 6 protease inhibitors and one HIV-1 fusion inhibitor) showed highly synergistic anti-HIV-1 activity. In these studies, no controls representative of combination effects (additive, synergistic or antagonistic) were included to evaluate the true effects of the combination study results.

At the request of the FDA the applicant provided their contractor supplied datasets as an electronic mail communication on the combination activity on December 7, 2004. In consideration of the large datasets submitted for review, shorter remaining time frame for evaluation of the submission prior to the NDA action date, lack of an official submission of the data from Roche and for lack of appropriate controls in these combination studies, the applicant is requested to conduct these combination activity studies and provide a detailed report.

MaxCmin-1 and MaxCmin-2 clinical studies: The Copenhagen HIV program (CHIP) with the financial support of Hoffmann-La Roche Inc. conducted these two Phase 4, randomized, open-label, multicenter trials that compared the safety and efficacy of ritonavir-boosted saquinavir in combination with other antiretroviral agents. In the MaxCmin-1 trial the safety and efficacy of the ritonavir-boosted indinavir (indinavir/ritonavir = 800/100 mg, bid) was compared with that of the ritonavir-boosted saquinavir (saquinavir/ritonavir = 1000/100 mg, bid) over 48 weeks in HIV-1 infected adults. In the MaxCmin-2 trial the safety and efficacy of the ritonavir-boosted lopinavir (lopinavir/ritonavir = 400/100 mg, bid) was compared with that of the ritonavir boosted-saquinavir (saquinavir/ritonavir = 1000/100 mg, bid), over 48 weeks in HIV-1 infected adults (for details of the clinical studies, please see the clinical review by Dr. Yoshi Murata).

The primary outcome in both of the trials was virologic failure. Virologic failure included observed virologic failure and non-observed virologic failure (death, lost-to-follow-up and withdrawing consent). The definition of observed virologic failure was slightly different between the two trials.

Virologic failure for MaxCmin-1 study:

1. For subjects entering the study with viral load of <200 copies/ml, an HIV-RNA value of ≥ 200 copies/ml.
2. For subjects entering the study with viral load of ≥ 200 copies/ml:
 - (a) Any rise in HIV-RNA of $\geq 0.5 \log_{10}$ copies/ml
 - (b) Viral load of $\geq 50,000$ copies/ml more than 5 weeks after baseline and or
 - (c) Viral load of $\geq 5,000$ copies/ml more than 14 weeks after baseline
 - (d) Viral load of ≥ 200 copies/ml more than 27 weeks after baseline

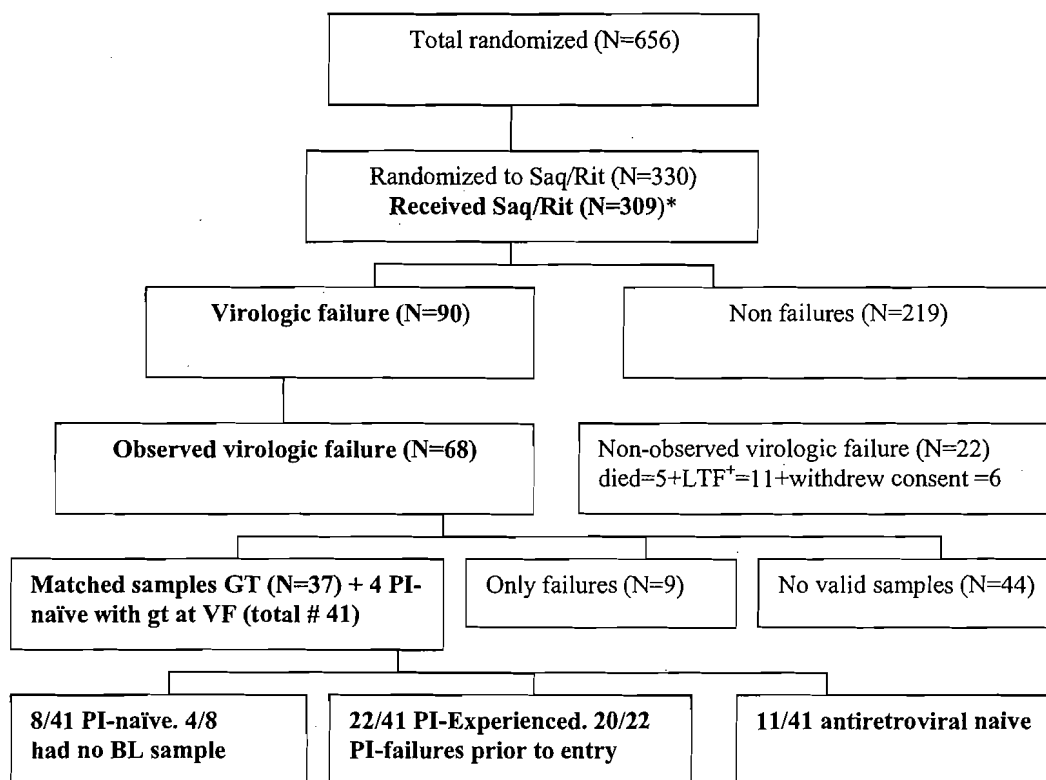
All cases of suspected virological failure should be confirmed by a second HIV-RNA determination performed at least 2 weeks later or as soon as possible. Once confirmed, the time of virologic failure is defined as the time of the first measurement.

Virologic failure for MaxCmin-2 study:

1. For subjects entering the study with viral load of <200 copies/ml, an HIV-RNA value of ≥ 200 copies/ml.
2. For subjects entering the study with viral load of ≥ 200 copies/ml:
 - (a) Any rise in HIV-RNA of $\geq 0.5 \log_{10}$ copies/ml and/or
 - (b) Viral load at week 4: $\leq 0.5 \log_{10}$ reduction from baseline if ≥ 200 copies/ml at week 4
 - (c) Viral load at week 12: $\leq 1.0 \log_{10}$ reduction from baseline if ≥ 200 copies/ml at week 12 or
 - (d) Viral load at week 24: ≥ 200 copies/ml at 24 weeks

All cases of suspected virological failure should be confirmed by a second HIV-RNA determination performed at least 2 weeks later or as soon as possible. Once confirmed, the time of virologic failure is defined as the time of the first measurement.

Chart showing the patient flow and disposition



*= Bolded text indicates patients who were treated with Saquinavir/ritonavir and followed for evaluation of resistance, + = Lost-to-follow-up

The applicant submitted genotypic data sets for virologic failure patients assigned to and started on saquinavir/ritonavir treatment arms of both MaxCmin-1 and MaxCmin-2 trials. In the genotypic analysis, only mutations occurring in the protease section of the *pol* gene were reported. In view of the similarities of both of the clinical studies with regard to their inclusion/exclusion criteria, enrolment and follow up procedures and virologic failure criteria, the virology datasets of the two clinical trials were pooled for analysis. Both of the trials included patients who were ART-experienced, PI-experienced and ART-naïve.

Frozen plasma samples from CHIP central plasma repository were used in the HIV-1 genotypic resistance analysis. The chart on patient flow and disposition shows that out of the 330 patients randomized to saquinavir/ritonavir, 309 patients received the saquinavir/ritonavir combination from a total of 656 patients randomized (intent-to-treat basis) in both of the MaxCmin-1 and MaxCmin-2 trials. Out of 68 with observed virologic failure, 37 patients had matched (baseline and treatment failure) samples. In addition, 4 protease-naïve patients without baseline samples but with therapy failure sample were included in this study (assuming that the PI-naïves harbored wild-type virus at baseline).

The applicant stated that out of the 41 virologic failure samples 11 were antiretroviral naïve, 8 were PI-naïve and 22 were PI-experienced. Of the 41 failure samples, 8 had mutations in their primary resistance profile. Virologic failure samples with changes to the PI-resistance profiles are shown in Table 3.

Table 3. Patients with changes in primary PI mutations profile

Patient	Baseline	Failure	PI-Experience		On treatment at failure
			Overall	SAQ	
1100213	L90M	I84V, L90M	Yes	No	Yes
1213214		M46I/M, V82A, L90M	Yes	No	Yes
	No sample	No data	No	No	Yes
1503115	V82A	V82A, I84I/V	Yes	No	Yes
1503202	No sample	No data	Yes	No	No
3003102	D30N, M46I	D30N, M46I, I84V	Yes	No	Yes
5502102		M46I/M	No	No	No
8500102	M46I/M, G48V, V82A/V, L90M	M46I/M, G48V, V82A/V, I84I/V, L90M	Yes	Yes	Yes

Roche stated that 8/41 (20%) patients showed changes to their resistance profile and 7/41 (17%) had evidence of the emergence of primary PI resistance. The data presented in Table 3 however showed PI-resistance data for only 6 patients. In addition, the data were not presented in the template format as requested. Therefore, the applicant has not fulfilled the Phase 4 commitment and is requested to provide the resistance data according to the requested format with additional details.

Conclusions and Recommendations: With regard to microbiology the application is recommended for approval.

Phase 4 considerations:

The sponsor has not fulfilled the previous Phase 4 commitments as the submitted data were incomplete. The sponsor need to:

Determine the baseline genotype of all PI-experienced responders in the MaxCmin studies 1 and 2 and submit the data in the resistance template format. Resubmit virologic failure data sets with columns identifying isolates (specifically baseline) and with column identifying outcome (nonresponder, rebound, censored etc.)

Timeframe for submission = On or before June 17, 2005

Microbiology portion of the Package Insert**MICROBIOLOGY****Mechanism of Action**

Saquinavir is an inhibitor of HIV protease. HIV protease is an enzyme required for the proteolytic cleavage of viral polyprotein precursors into individual functional proteins found in infectious HIV. Saquinavir is a peptide-like substrate analogue that binds to the protease active site and inhibits the activity of the enzyme. Saquinavir inhibition prevents cleavage of the viral polyproteins resulting in the formation of immature noninfectious virus particles.

Antiviral Activity

In vitro antiviral activity of saquinavir was assessed in lymphoblastoid and monocytic cell lines and in peripheral blood lymphocytes. Saquinavir inhibited HIV activity in both acutely and chronically infected cells. IC₅₀ and IC₉₀ values (50% and 90% inhibitory concentrations) were in the range of 1 to 30 nM and 5 to 80 nM, respectively. In the presence of 40% human serum, the mean IC₅₀ of saquinavir against laboratory strain HIV-1 RF in MT4 cells was 37.7±5nM representing a 4-fold increase in the IC₅₀ value. In cell culture, saquinavir demonstrated additive to synergistic effects against HIV-1 in combination with reverse transcriptase inhibitors (didanosine, lamivudine, nevirapine, stavudine, zalcitabine and zidovudine) without enhanced cytotoxicity. Saquinavir in combination with the protease inhibitors amprenavir, atazanavir, or lopinavir resulted in synergistic antiviral activity. Saquinavir displayed antiviral activity in vitro against HIV-1 clades A-H (IC₅₀ values ranged from 0.9 to 2.5 nM). The IC₅₀ and IC₉₀ values of saquinavir against HIV-2 isolates in vitro ranged from 0.25 nM to 14.6 nM and 4.65 nM to 28.6 nM, respectively.

Drug Resistance

HIV-1 mutants with reduced susceptibility to saquinavir have been selected during in vitro passage. Genotypic analyses of these isolates showed several substitutions in the

HIV protease gene. Only the G48V and L90M substitutions were associated with reduced susceptibility to saquinavir, and conferred an increase in the IC₅₀ value of 8- and 3-fold, respectively.

HIV-1 isolates with reduced susceptibility (≥ 4 -fold increase in the IC₅₀ value) to saquinavir emerged in some patients treated with INVIRASE. Genotypic analysis of these isolates identified resistance conferring primary mutations in the protease gene G48V and L90M, and secondary mutations L10I/R/V, I54V/L, A71V/T, G73S, V77I, V82A and I84V that contributed additional resistance to saquinavir. Forty-one isolates from 37 patients failing therapy with INVIRASE had a median decrease in susceptibility to saquinavir of 4.3 fold.

The degree of reduction in in vitro susceptibility to saquinavir of clinical isolates bearing substitutions G48V and L90M depends on the number of secondary mutations present. In general, higher levels of resistance are associated with greater number of mutations only in association with either or both of the primary mutations G48V and L90M. No data are currently available to address the development of resistance in patients receiving saquinavir/ritonavir.

Cross-resistance

Among protease inhibitors, variable cross-resistance has been observed. In one clinical study, 22 HIV-1 isolates with reduced susceptibility (> 4 -fold increase in the IC₅₀ value) to saquinavir following therapy with INVIRASE were evaluated for cross-resistance to amprenavir, indinavir, nelfinavir and ritonavir. Six of the 22 isolates (27%) remained susceptible to all 4 protease inhibitors, 12 of the 22 isolates (55%) retained susceptibility to at least one of the PIs and 4 out of the 22 isolates (18%) displayed broad cross-resistance to all PIs. Sixteen (73%) and 11 (50%) of the 22 isolates remained susceptible (< 4 -fold) to amprenavir and indinavir, respectively. Four of 16 (25%) and nine of 21 (43%) with available data remained susceptible to nelfinavir and ritonavir, respectively.

After treatment failure with amprenavir, cross-resistance to saquinavir was evaluated. HIV-1 isolates from 22/22 patients failing treatment with amprenavir and containing one or more mutations M46L/I, I50V, I54L, V32I, I47V, and I84V were susceptible to saquinavir.

Narayana Battula, Ph.D.
Microbiologist

Concurrence:

HFD 530/ Dir. _____ Date _____

HFD 530/TLMicro. _____ Date _____

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Narayana Battula
12/16/04 03:54:18 PM
MICROBIOLOGIST

Micro review for Saquinavir film coated tablet, NDA 21-785

Julian O Rear
12/16/04 04:32:45 PM
MICROBIOLOGIST

Debra Birnkrant
12/17/04 10:49:46 AM
MEDICAL OFFICER