NDA

20628

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

NDA 20-628

DEC 6 1995

Hoffmann-La Roche
Attn: Mary Ellen Mulligan
Director, Regulatory Affairs
340 Kingsland Street
Nutley, New Jersey 07110-1199

Dear Ms. Mulligan:

Please refer to your August 31, 1995, New Drug Applications (NDA) submitted pursuant to section 505 (b) of the Federal Food, Drug, and Cosmetic Act for Invirase (saquinavir mesylate) capsules.

We acknowledge receipt of your amendments dated:

September 15, 1995	October 4, 1995	October 26, 1995
September 21,1995	October 5, 1995	November 20, 1995
September 26, 1995	October 9, 1995	December 6, 1995 (3)
September 27, 1995	October 23, 1995	
September 28, 1995 (2)	October 24, 1995 (2)	
September 29, 1995 (2)	October 25, 1995	

This new drug application is indicated for use in combination with nucleoside analogues for the treatment of advanced HIV infection in selected patients.

We have completed our review of these applications and have concluded that adequate information has been presented to demonstrate that the drug product is safe and effective for use as recommended in the final printed label submitted December 6, 1995. Accordingly, this application is approved effective on the date of this letter.

We acknowledge your commitment to comply with the conditions of Accelerated Approval as stated in your December 6, 1995 letter. Additionally, we acknowledge your commitment to conduct the phase 4 studies stated in your December 6, 1995 letter.

Please submit 20 copies of the FPL as soon as available. Please individually mount ten of the copies on heavy weight paper or similar material. For administrative purposes, this submission should be designated "FINAL PRINTED LABELING for approved NDA 20-628." Approval of this labeling is not required before it is used.

Should additional information relating to the safety and effectiveness of the drug become available, revision of that labeling may be required.

Validation of the regulatory methods has not been completed. At the present time, it is the policy of the Center not to withhold approval because the methods are being validated. Nevertheless, we expect your continued cooperation to resolve any deficiencies that may occur.

Please submit one market package of the drug when it is available.

Under section 736(a) (1) (B) (ii) of the Prescription Drug User Fee Act of 1992, this letter triggers the remaining 50% of the fee assessed for this application. You will receive an invoice for the amount due within the next month. Payment will be due within 30 days of the date of the invoice.

We remind you that you must comply with the requirements for an approved NDA set forth under 21 CFR 314.80 and 314.81.

If you have any questions, please contact Vikki S. Kinsey, Consumer Safety Officer at 301-827-2335

Sincerely yours,

David W. Feigal, Jr., M.D., M.P.H.
Director
Division of Antiviral Drug Products
Office of Drug Evaluation II
Center for Drug Evaluation and Research

(Roche Hexagon)

INVIRASE™

(saquinavir mesylate)

CAPSULES

WARNING: The indication for INVIRASE for the treatment of HIV infection is based on changes in surrogate markers. At present there are no results from controlled clinical trials evaluating the effect of regimens containing INVIRASE on survival or the clinical progression of HIV infection, such as the occurrence of opportunistic infections or malignancies.

DESCRIPTION: INVIRASE brand of saquinavir mesylate is an inhibitor of the human immunodeficiency virus (HIV) protease. INVIRASE is available as light brown and green, opaque hard gelatin capsules for oral administration in a 200-mg strength (as saquinavir free base). Each capsule also contains the inactive ingredients lactose, microcrystalline cellulose, povidone K30, sodium starch glycolate, talc and magnesium stearate. Each capsule shell contains gelatin and water with the following dye systems: red iron oxide, yellow iron oxide, black iron oxide, FD&C Blue #2 and titanium dioxide. The chemical name for saquinavir mesylate is N-tert-butyl-decahydro-2-[2(R)-hydroxy-4-phenyl-3(S)-[[N-(2-quinolylcarbonyl)-L-asparaginyl]amino]butyl]-(4aS,8aS)-isoquinoline-3(S)-carboxamide methanesulfonate with a molecular formula $C_{38}H_{50}N_6O_5 \cdot CH_4O_3S$ and a molecular weight of 766.96. The molecular weight of the free base is 670.86. Saquinavir mesylate has the following structural formula:

INVIRASE™ (saquinavir mesylate)

Saquinavir mesylate is a white to off-white, very fine powder with an aqueous solubility of 2.22 mg/mL at 25°C.

CLINICAL PHARMACOLOGY: ***Mechanism of Action:*** HIV protease cleaves viral polyprotein precursors to generate functional proteins in HIV-infected cells. The cleavage of viral polyprotein precursors is essential for maturation of infectious virus. Saquinavir mesylate, henceforth referred to as saquinavir, is a synthetic peptide-like substrate analogue that inhibits the activity of HIV protease and prevents the cleavage of viral polyproteins.

Microbiology: Antiviral Activity In Vitro The 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 values (50% inhibitory concentration) were in the range of 1 to 30 nM. In cell culture saquinavir demonstrated additive to synergistic effects against HIV in double and triple combination regimens with reverse transcriptase inhibitors zidovudine (ZDV), zalcitabine (ddC) and didanosine (ddI), without enhanced cytotoxicity.

Resistance HIV isolates with reduced susceptibility to saquinavir have been selected in vitro. Genotypic analyses of these isolates showed substitution mutations in the HIV protease at amino acid positions 48 (Glycine to Valine) and 90 (Leucine to Methionine).

Phenotypic and genotypic changes in HIV isolates from patients treated with saquinavir were

also monitored in Phase 1/2 clinical trials. Phenotypic changes were defined as a tenfold decrease in sensitivity from baseline. Two viral protease mutations (L90M and/or G48V, the former predominating) were found in virus from treated, but not untreated, patients. The incidence across studies of phenotypic and genotypic changes in the subsets of patients studied for a period of 16 to 74 weeks (median observation time approximately 1 year) is shown in Table 1. However, the clinical relevance of phenotypic and genotypic changes associated with saquinavir therapy has not been established.

Cross-resistance to Other Antiretrovirals: The potential for HIV cross-resistance between protease inhibitors has not been fully explored. Therefore, it is unknown what effect saquinavir therapy will have on the activity of subsequent protease inhibitors. Cross-resistance between saquinavir and reverse transcriptase inhibitors is unlikely because of the different enzyme targets involved. ZDV-resistant HIV isolates have been shown to be sensitive to saquinavir in vitro.

Pharmacokinetics: The pharmacokinetic properties of saquinavir have been evaluated in healthy volunteers (n=351) and HIV-infected patients (n=270) after single and multiple oral doses of 25, 75, 200 and 600 mg tid and in healthy volunteers after intravenous doses of 6, 12, 36 or 72 mg (n=21).

Absorption and Bioavailability in Adults: Following multiple dosing (600 mg tid) in HIV-infected patients (n=29), the steady-state area under the plasma concentration versus time curve (AUC) was 2.5 times (95% CI 1.6 to 3.8) higher than that observed after a single dose.

INVIRASE™ (saquinavir mesylate)

HIV-infected patients administered saquinavir 600 mg tid. with the instructions to take saquinavir after a meal or substantial snack, had AUC and maximum plasma concentration (Cmax) values which were about twice those observed in healthy volunteers receiving the same treatment regimen (Table 2).

Absolute bioavailability averaged 4% (CV 73%, range 1% to 9%) in 8 healthy volunteers who received a single 600 mg dose (3 x 200 mg) of saquinavir following a high fat breakfast (48 g protein, 60 g carbohydrate, 57 g fat, 1006 kcal). The low bioavailability is thought to be due to a combination of incomplete absorption and extensive first-pass metabolism.

Food Effect The mean 24-hour AUC after a single 600 mg oral dose (6 x 100 mg) in healthy volunteers (n=6) was increased from 24 ng h/mL (CV 33%), under fasting conditions, to 161 ng h/mL (CV 35%) when saquinavir was given following a high fat breakfast (48 g protein, 60 g carbohydrate, 57 g fat, 1006 kcal). Saquinavir 24-hour AUC and Cmax (n=6) following the administration of a higher calorie meal (943 kcal, 54 g fat) were on average two times higher than after a lower calorie, lower fat meal (355 kcal, 8 g fat). The effect of food has been shown to persist for up to 2 hours.

Distribution in Adults The mean steady-state volume of distribution following intravenous administration of a 12-mg dose of saquinavir (n=8) was 700 L (CV 39%), suggesting saquinavir partitions into tissues. Saquinavir was approximately 98% bound to plasma proteins over a concentration range of 15 to 7000 ng/mL. In 2 patients receiving saquinavir 600 mg tid, cerebrospinal fluid concentrations were negligible when compared to concentrations from

matching plasma samples

Metabolism and Elimination in Adults In vitro studies using human liver microsomes have shown that the metabolism of saquinavir is cytochrome P450 mediated with the specific isoenzyme, CYP3A4, responsible for more than 90% of the hepatic metabolism. Based on in vitro studies, saquinavir is rapidly metabolized to a range of mono- and di-hydroxylated inactive compounds. In a mass balance study using 600 mg ^{14}C-saquinavir (n=8), 88% and 1% of the orally administered radioactivity was recovered in feces and urine, respectively, within 48 hours of dosing. In an additional 4 subjects administered 10.5 mg 14C-saquinavir intravenously, 81% and 3% of the intravenously administered radioactivity was recovered in feces and urine, respectively, within 48 hours of dosing. In mass balance studies, 13% of circulating radioactivity in plasma was attributed to unchanged drug after oral administration and the remainder attributed to saquinavir metabolites. Following intravenous administration, 66% of circulating radioactivity was attributed to unchanged drug and the remainder attributed to saquinavir metabolites, suggesting that saquinavir undergoes extensive first-pass metabolism.

Systemic clearance of saquinavir was rapid (1.13 L/h/kg (CV 12%) after intravenous doses of 6, 36 and 72 mg. The mean residence time of saquinavir was 7 hours (n=8).

Special Populations: Hepatic or Renal Impairment: Saquinavir pharmacokinetics in patients with hepatic or renal insufficiency has not been investigated (see PRECAUTIONS).

Gender, Race and Age Pharmacokinetic data were available for 17 women in the Phase 1/2 studies. Pooled data did not reveal an apparent effect of gender on the pharmacokinetics of saquinavir.

The effect of race on the pharmacokinetics of saquinavir has not been evaluated, due to the small numbers of minorities for whom pharmacokinetic data were available.

Saquinavir pharmacokinetics has not been investigated in patients >65 years of age or in pediatric patients (<16 years).

Drug Interactions: HIVID and ZDV Concomitant use of INVIRASE with HIVID® (zalcitabine, ddC) and ZDV has been studied (as triple combination) in adults. Pharmacokinetic data suggest that the absorption, metabolism and elimination of each of these drugs are unchanged when they are used together.

Ketoconazole Concomitant administration of ketoconazole (200 mg qd) and saquinavir (600 mg tid) to 12 healthy volunteers resulted in steady-state saquinavir AUC and Cmax values which were three times those seen with saquinavir alone. No dose adjustment is required when the two drugs are coadministered at the doses studied. Ketoconazole pharmacokinetics was unaffected by coadministration with saquinavir.

Rifampin Coadministration of rifampin (600 mg qd) and saquinavir (600 mg tid) to 12 healthy volunteers decreased the steady-state AUC and Cmax of saquinavir by approximately

80%.

Rifabutin: Preliminary data from 12 HIV-infected patients indicate that the steady-state AUC of saquinavir (600 mg tid) was decreased by 40% when saquinavir was coadministered with rifabutin (300 mg qd).

INDICATIONS AND USAGE: INVIRASE in combination with nucleoside analogues is indicated for the treatment of advanced HIV infection in selected patients (see *Description of Clinical Studies* below). This indication is based on changes in surrogate markers in patients who initiated INVIRASE concomitantly with either ZDV (in previously untreated patients) or HIVID (in patients previously treated with prolonged zidovudine therapy). At present, there are no results available from trials evaluating the activity of INVIRASE in combination with nucleoside analogues other than ZDV or HIVID. There are also no results available from clinical trials confirming the clinical benefit of combination therapy with INVIRASE on HIV disease progression or survival.

Description of Clinical Studies: The activity of INVIRASE in combination with HIVID and/or ZDV in HIV infection has been evaluated in three double-blind, randomized trials in a total of 810 patients with advanced HIV infection.

Advanced Patients without Prior ZDV Therapy: A dose-ranging study (Italy, V13330) conducted in 92 ZDV-naive patients (mean baseline CD_4=179) studied INVIRASE at doses of

75 mg, 200 mg and 600 mg tid in combination with ZDV 200 mg tid compared to INVIRASE 600 mg tid alone and ZDV alone

In analyses of average CD_4 changes over 16 weeks, treatment with the combination of INVIRASE 600 mg tid + ZDV produced greater CD_4 cell increases than ZDV monotherapy (see Fig. 1). The CD_4 changes of ZDV in combination with doses of INVIRASE lower than 600 mg tid were no greater than that of ZDV alone

Advanced Patients with Prior ZDV Therapy In ACTG229/NV14255; 295 patients (mean baseline CD4=165) with prolonged ZDV treatment (median 713 days) were randomized to receive either INVIRASE 600 mg tid + HIVID + ZDV (triple combination), INVIRASE 600 mg tid + ZDV or HIVID + ZDV. In analyses of average CD_4 changes over 24 weeks, the triple combination produced greater increases in CD_4 cell counts (see Fig. 2) compared to that of HIVID + ZDV. There were no significant differences in CD_4 changes among patients receiving INVIRASE + ZDV and HIVID + ZDV

Study NV14256 (North America) is an ongoing, randomized, double-blind study comparing INVIRASE 600 mg tid + HIVID to HIVID monotherapy and INVIRASE monotherapy in patients with advanced HIV infection and at least 16 weeks of prior ZDV treatment. The study remains blinded with respect to clinical endpoints of disease progression, however, analyses of CD4 changes over 16 weeks were conducted for a cohort of 423 patients. These analyses showed that the combination of INVIRASE + HIVID was associated with greater CD_4 increases than either HIVID or INVIRASE as monotherapy (see Fig. 3)

Comparisons of data across studies (NV14256 compared to ACTG229/NV14255) suggest that when INVIRASE was added to a regimen of prolonged prior zidovudine, there was little activity contributed by continuing ZDV.

HIV RNA. The clinical significance of changes in HIV-RNA measurements have not been established. At present, this laboratory measure is available on an experimental basis to monitor antiviral activity in clinical trials. Table 3 compares log RNA reductions at 16 weeks among INVIRASE combination treatment arms in three clinical trials. Monotherapy arms are included for reference. Overall, RNA reductions were greater in INVIRASE/nucleoside combination regimens compared to nucleoside monotherapy controls.

CONTRAINDICATIONS: INVIRASE is contraindicated in patients with clinically significant hypersensitivity to saquinavir or to any of the components contained in the capsule.

PRECAUTIONS: ***General***. The safety profile of INVIRASE in children younger than 16 years has not been established.

If a serious or severe toxicity occurs during treatment with INVIRASE, INVIRASE should be interrupted until the etiology of the event is identified or the toxicity resolves. At that time, resumption of treatment with full dose INVIRASE may be considered. For nucleoside analogues used in combination with INVIRASE, physicians should refer to the complete product information for these drugs for dose adjustment recommendations and for information

regarding drug-associated adverse reactions

Caution should be exercised when administering INVIRASE to patients with hepatic insufficiency since patients with baseline liver function tests >5 times the upper limit of normal were not included in clinical studies

Resistance/Cross-resistance: The potential for HIV cross-resistance between protease inhibitors has not been fully explored. Therefore, it is unknown what effect saquinavir therapy will have on the activity of subsequent protease inhibitors (see *Microbiology*)

Information for Patients: Patients should be informed that INVIRASE is not a cure for HIV infection and that they may continue to acquire illnesses associated with advanced HIV infection, including opportunistic infections. INVIRASE has not been shown to reduce the incidence or frequency of such illnesses, and patients should be advised to remain under the care of a physician while using INVIRASE

Patients should be told that the long-term effects of INVIRASE are unknown at this time. They should be informed that INVIRASE therapy has not been shown to reduce the risk of transmitting HIV to others through sexual contact or blood contamination.

Patients should be advised that INVIRASE should be taken within 2 hours after a full meal (see *Pharmacokinetics*). When INVIRASE is taken without food, concentrations of saquinavir in the blood are substantially reduced and may result in no antiviral activity.

Laboratory Tests: No consistent alterations in standard laboratory tests have been associated with the use of INVIRASE. Clinical chemistry tests should be performed prior to initiating INVIRASE therapy and at appropriate intervals thereafter. For comprehensive information concerning laboratory test alterations associated with use of individual nucleoside analogues, physicians should refer to the complete product information for these drugs.

Drug Interactions: Metabolic Enzyme Inducers INVIRASE should not be administered concomitantly with rifampin, since rifampin decreases saquinavir concentrations by 80% (see *Pharmacokinetics*). Rifabutin also substantially reduces saquinavir plasma concentrations by 40%. Other drugs that induce CYP3A4 (eg, phenobarbital, phenytoin, dexamethasone, carbamazepine) may also reduce saquinavir plasma concentrations. If therapy with such drugs is warranted, physicians should consider using alternatives when a patient is taking INVIRASE.

Other Potential Interactions Coadministration of terfenadine or astemizole with drugs that are known to be potent inhibitors of the cytochrome P4503A pathway (ie, ketoconazole, itraconazole, etc.) may lead to elevated plasma concentrations of terfenadine or astemizole, which may in turn prolong QT intervals leading to rare cases of serious cardiovascular adverse events. Although INVIRASE is not a strong inhibitor of cytochrome P4503A, pharmacokinetic interaction studies with INVIRASE and terfenadine or astemizole have not been conducted. Physicians should use alternatives to terfenadine or astemizole when a patient taking INVIRASE requires antihistamines. Other compounds that are substrates of CYP3A4 (eg, calcium channel blockers, clindamycin, dapsone, quinidine, triazolam) may have elevated

plasma concentrations when coadministered with INVIRASE, therefore, patients should be monitored for toxicities associated with such drugs

Carcinogenesis, Mutagenesis and Impairment of Fertility: Carcinogenesis: Carcinogenicity studies in rats and mice have not yet been completed

Mutagenesis: Mutagenicity and genotoxicity studies, with and without metabolic activation where appropriate, have shown that saquinavir has no mutagenic activity in vitro in either bacterial (Ames test) or mammalian cells (Chinese hamster lung V79/HPRT test). Saquinavir does not induce chromosomal damage in vivo in the mouse micronucleus assay or in vitro in human peripheral blood lymphocytes and does not induce primary DNA damage in vitro in the unscheduled DNA synthesis test

Impairment of Fertility: Fertility and reproductive performance were not affected in rats at plasma exposures (AUC values) up to five times those achieved in humans at the recommended dose

Pregnancy: Teratogenic Effects: Category B Reproduction studies conducted with saquinavir in rats have shown no embryotoxicity or teratogenicity at plasma exposures (AUC values) up to five times those achieved in humans at the recommended dose or in rabbits at plasma exposures four times those achieved at the recommended clinical dose. Studies in rats indicated that exposure to saquinavir from late pregnancy through lactation at plasma concentrations (AUC values) up to five times those achieved in humans at the recommended

dose had no effect on the survival, growth and development of offspring to weaning. Because animal reproduction studies are not always predictive of human response, INVIRASE should be used during pregnancy after taking into account the importance of the drug to the mother. Presently, there are no reports of infants being born after women receiving INVIRASE in clinical trials became pregnant.

Nursing Mothers: It is not known whether INVIRASE is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from saquinavir, a decision should be made whether to discontinue nursing or discontinue the drug, taking into account the importance of INVIRASE to the mother.

Pediatric Use: Safety and effectiveness of INVIRASE in HIV-infected children or adolescents younger than 16 years of age have not been established.

ADVERSE REACTIONS (see PRECAUTIONS) The safety of INVIRASE was studied in 688 patients who received the drug either alone or in combination with ZDV and/or HIVID (zalcitabine, ddC). The majority of adverse events were of mild intensity. The most frequently reported adverse events among patients receiving INVIRASE (excluding those toxicities known to be associated with ZDV and HIVID when used in combinations) were diarrhea, abdominal discomfort and nausea.

INVIRASE did not alter the pattern, frequency or severity of known major toxicities

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associated with the use of HIVID and/or ZDV. Physicians should refer to the complete product information for these drugs (or other antiretroviral agents as appropriate) for drug-associated adverse reactions to other nucleoside analogues.

Table 4 lists clinical adverse events that occurred in ≥2% of patients receiving INVIRASE 600 mg tid alone or in combination with ZDV and/or HIVID in two trials. Median duration of treatment in NV14255/ACTG229 (triple combination study) was 48 weeks; median duration of treatment among the surrogate analysis cohort analyzed for safety (n=451) in NV14256 was 42 weeks.

Rare occurrences of the following serious adverse experiences have been reported during clinical trials of INVIRASE and were considered at least possibly related to use of study drugs: confusion, ataxia and weakness; acute myeloblastic leukemia; hemolytic anemia; attempted suicide; Stevens-Johnson syndrome; seizures; severe cutaneous reaction associated with increased liver function tests; isolated elevation of transaminases; thrombophlebitis; headache and thrombocytopenia; exacerbation of chronic liver disease with Grade 4 elevated liver function tests; jaundice; ascites; and right and left upper quadrant abdominal pain.

Table 5 shows the percentage of patients with marked laboratory abnormalities in studies NV14255/ACTG229 and NV14256. Marked laboratory abnormalities are defined as a Grade 3 or 4 abnormality in a patient with a normal baseline value or a Grade 4 abnormality in a patient with a Grade 1 abnormality at baseline (ACTG Grading System).

Monotherapy and Combination Studies: Other clinical adverse experiences of any intensity, at least remotely related to INVIRASE, including those in <2% of patients on arms containing INVIRASE in studies NV14255/ACTG229 and NV14256, and those in smaller clinical trials, are listed below by body system

Body as a Whole: Allergic reaction, chest pain, edema, fever, intoxication, parasites external, retrosternal pain, shivering, wasting syndrome, weight decrease

Cardiovascular: Cyanosis, heart murmur, heart valve disorder, hypertension, hypotension, syncope, vein distended

Endocrine/Metabolic: Dehydration, dry eye syndrome, hyperglycemia, weight increase, xerophthalmia

Gastrointestinal: Cheilitis, constipation, dysphagia, eructation, feces bloodstained, feces discolored, gastralgia, gastritis, gastrointestinal inflammation, gingivitis, glossitis, hemorrhage rectum, hemorrhoids, hepatomegaly, hepatosplenomegaly, melena, pain pelvic, painful defecation, pancreatitis, parotid disorder, salivary glands disorder, stomatitis, tooth disorder, vomiting

Hematologic: Anemia, microhemorrhages, pancytopenia, splenomegaly, thrombocytopenia

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Musculoskeletal: Arthralgia, arthritis, back pain, cramps muscle, musculoskeletal disorders, stiffness, tissue changes, trauma

Neurological: Ataxia, bowel movements frequent, confusion, convulsions, dysarthria, dysesthesia, heart rate disorder, hyperesthesia, hyperreflexia, hyporeflexia, mouth dry, numbness face, pain facial, paresis, poliomyelitis, progressive multifocal leukoencephalopathy, spasms, tremor

Psychological: Agitation, amnesia, anxiety, depression, dreaming excessive, euphoria, hallucination, insomnia, intellectual ability reduced, irritability, lethargy, libido disorder, overdose effect, psychic disorder, somnolence, speech disorder

Reproductive System: Prostate enlarged, vaginal discharge

Resistance Mechanism: Abscess, angina tonsillaris, candidiasis, hepatitis, herpes simplex, herpes zoster, infection bacterial, infection mycotic, infection staphylococcal, influenza, lymphadenopathy, tumor

Respiratory: Bronchitis, cough, dyspnea, epistaxis, hemoptysis, laryngitis, pharyngitis, pneumonia, respiratory disorder, rhinitis, sinusitis, upper respiratory tract infection

Skin and Appendages: Acne, dermatitis, dermatitis seborrheic, eczema, erythema, folliculitis,

furunculosis, hair changes, hot flushes, photosensitivity reaction, pigment changes skin, rash maculopapular, skin disorder, skin nodule, skin ulceration, sweating increased, urticaria, verruca, xeroderma

Special Senses: Blepharitis, earache, ear pressure, eye irritation, hearing decreased, otitis, taste alteration, tinnitus, visual disturbance

Urinary System: Micturition disorder, urinary tract infection

OVERDOSAGE: No acute toxicities or sequelae were noted in 1 patient who ingested 8 grams of INVIRASE as a single dose. The patient was treated with induction of emesis within 2 to 4 hours after ingestion. In an exploratory Phase 2 study of oral dosing with INVIRASE at 7200 mg/day (1200 mg q4h), there were no serious toxicities reported through the first 25 weeks of treatment.

DOSAGE AND ADMINISTRATION: The recommended dose for INVIRASE in combination with a nucleoside analogue is three 200-mg capsules three times daily taken within 2 hours after a full meal. The recommended doses of HIVID (zalcitabine, ddC) or ZDV as part of combination therapy are HIVID 0.75 mg three times daily or ZDV, 200 mg three times daily as appropriate.

Monitoring of Patients: Clinical chemistry tests should be performed prior to initiating INVIRASE therapy and at appropriate intervals thereafter. For comprehensive patient

monitoring recommendations for other nucleoside analogues, physicians should refer to the complete product information for these drugs

Dose Adjustment for Combination Therapy with INVIRASE For toxicities that may be associated with INVIRASE, the drug should be interrupted INVIRASE at doses less than 600 mg tid are not recommended since lower doses have not shown antiviral activity For recipients of combination therapy with INVIRASE and nucleoside analogues, dose adjustment of the nucleoside analogue should be based on the known toxicity profile of the individual drug Physicians should refer to the complete product information for these drugs for comprehensive dose adjustment recommendations and drug-associated adverse reactions of nucleoside analogues

HOW SUPPLIED: INVIRASE 200 mg capsules are light brown and green opaque capsules with ROCHE and 0245 imprinted on the capsule shell - bottles of 270 (NDC 0004-0245-15).

The capsules should be stored at 59° to 86°F (15° to 30°C) in tightly closed bottles.

Manufactured by F Hoffmann La Roche

& Co Ltd , Basle, Switzerland or

Hoffmann-La Roche Inc Nutley New Jersey

Distributed by

INVIRASE™ (saquinavir mesylate)

(Roche Hexagon)

Roche Laboratories

A Member of the Roche Group

Hoffmann-La Roche Inc
340 Kingsland Street
Nutley, New Jersey 07110-1199

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Table 1. Frequency of Genotypic and Phenotypic Changes in Selected Patients Treated with Saquinavir

	Genotypic*	Phenotypic†
Monotherapy	15/33 (45%)	5/11 (45%)
Combination Therapy	16/52 (31%)	11/29 (38%)

*Double mutation (G48V and L90M) has occurred in 2 of 33 patients receiving monotherapy.
†For some patients genotypic and phenotypic changes were unrelated.

Table 2. Mean (%CV) AUC and Cmax in Patients and Healthy Volunteers

	AUC_τ (dose interval) (ng h/mL)	Cmax (ng/mL)
Healthy Volunteers (n=6)	359.0 (46)	90.39 (49)
Patients (n=113)	757.2 (84)	253.3 (99)

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Table 3. Summary of Mean Log_{10} Plasma RNA Results from Major INVIRASE Clinical Studies*

	V13330(Italy) Naive patients			NV14255/ACTG229 (USA) ZDV-experienced			Surrogate Marker Analysis NV14256 (North America)		
	ZDV	CAQ[1]	ZDV+SAQ[1]	ZDV+ddC	ZDV+SAQ[1]	ZDV+ddC+SAQ[1]	ddC	SAQ[1]	SAQ[1]+ddC
n Enrolled	17	19	20	100	99	98	145	159	147
Prior ZDV									
n				99	98	97	134	151	136
Median Duration (days)				659	713	647	614	459	442
Log_{10} Plasma RNA by PCR (copies/mL)									
n	17	19	20	100	97	96	114	124	119
Mean Baseline (n)	5.2 (13)	5.2 (15)	5.2 (15)	4.7 (100)	4.8 (97)	4.8 (96)	5.2 (114)	5.1 (124)	5.1 (119)
Mean Change from Baseline Week 16	0.6	0.2	1.0	0.4	0.0	0.5	0.4	0.1	0.6
Mean Change from Baseline Week 24				0.2	0.0	0.5			

*NOTE: THE CLINICAL SIGNIFICANCE OF CHANGES IN HIV VIRAL RNA DURING THERAPY IS UNKNOWN

[1]Saquinavir (SAQ) at 600 mg tid

– Indicates not applicable

Table 4. Percentage of Patients, by Study Arm, with Clinical Adverse Experiences Considered at Least Possibly Related to Study Drug or of Unknown Relationship and of Moderate, Severe or Life-threatening Intensity, Occurring in ≥2% of Patients in NV14255/ACTG229 and NV14256

	NV14255/ACTG229			NV14256		
ADVERSE EVENT	SAQ+ZDV n=99	SAQ+ddC+ZDV n=98	ddC+ZDV n=100	ddC n=145	SAQ n=159	SAQ+ddC n=147
GASTROINTESTINAL						
Diarrhea	3.0	1.0	-	1.4	3.8	3.4
Abdominal Discomfort	2.0	3.1	4.0	1.4	1.3	0.7
Nausea	-	3.1	3.0	0.7	1.9	0.7
Dyspepsia	1.0	1.0	2.0	2.1	-	0.7
Abdominal Pain	2.0	1.0	2.0	0.7	1.9	0.7
Mucosa Damage	-	-	4.0	1.4	-	0.7
Buccal Mucosa Ulceration	-	2.0	2.0	9.0	2.5	4.1
CENTRAL AND PERIPHERAL NERVOUS SYSTEM						
Headache	2.0	2.0	2.0	4.1	0.6	0.7
Paresthesia	2.0	3.1	4.0	0.7	1.0	1.0
Extremity Numbness	2.0	1.0	4.0	-	-	0.7
Dizziness	-	2.0	1.0	-	-	-
Peripheral Neuropathy	-	1.0	2.0	5.5	-	4.8
BODY AS A WHOLE						
Asthenia	6.1	9.2	10.0	0.7	1.3	0.7
Appetite Disturbances	-	1.0	2.0	-	-	-
SKIN AND APPENDAGES						
Rash	-	-	3.0	0.7	1.3	1.4
Pruritus	-	-	2.0	-	-	-
MUSCULOSKELETAL DISORDERS						
Musculoskeletal Pain	2.0	2.0	4.0	-	0.6	0.7
Myalgia	1.0	-	3.0	1.4	-	-

-Indicates no events reported

Table 5. Percentage of Patients, by Treatment Group, with Marked Laboratory Abnormalities* in NV14255/ACTG229 and NV14256

	NV14255/ACTG229			NV14256		
	SAQ+ZDV n=99	SAQ+ZDV+ddC n=98	ZDV+ddC n=100	ddC n=145	SAQ n=159	SAQ+ddC n=147
BIOCHEMISTRY						
Calcium (high)	1	0	0	<1	0	0
Creatine Phosphokinase	10	12	7	6	4	7
Glucose (low)	0	0	0	4	5	4
Glucose (high)	0	0	0	0	<1	<1
Phosphorus	2	1	0	0	0	0
Potassium (high)	0	0	0	1	<1	<1
Potassium (low)	0	0	0	0	<1	0
Serum Amylase	2	1	1	<1	<1	2
SGOT (AST)	2	2	0	3	<1	<1
SGPT (ALT)	0	3	1	3	<1	<1
Total Bilirubin	1	0	0	0	<1	0
Uric Acid	0	0	1	Not assessed	Not assessed	Not assessed
HEMATOLOGY						
Neutrophils (low)	2	2	8	0	0	0
Hemoglobin (low)	0	0	1	0	<1	0
Platelets (low)	0	0	2	0	0	<1

*Marked Laboratory Abnormality defined as a shift from Grade 0 to at least Grade 3 from Grade 1 to Grade 4 (ACTG Grading System)

Roche

INVIRASE™

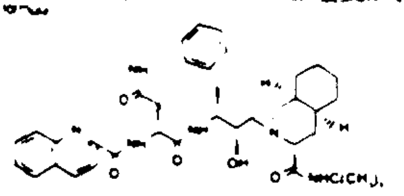
(saquinavir mesylate)

CAPSULES

BEST POSSIBLE COPY

> **WARNING:** The [illegible] for INVIRASE [illegible] for the [illegible] of HIV infection is based on changes in surrogate markers. At present there are no [illegible] controlled clinical trials evaluating the effect of regimens containing INVIRASE on [illegible] clinical progression of HIV infection, such as the occurrence of [illegible] or survival.

DESCRIPTION: INVIRASE [illegible] is an inhibitor of the human immunodeficiency virus (HIV) protease. INVIRASE is available as [illegible] and green opaque hard gelatin capsules for oral administration in a [illegible] mg strength [illegible] saquinavir free base. Each capsule also contains the inactive ingredients [illegible] povidone K30, sodium starch glycolate, talc and magnesium stearate. [illegible] contains gelatin and water with the following dye systems: [illegible] red iron oxide, FD&C Blue #2 and titanium dioxide. The chemical name for saquinavir mesylate is [illegible] (2R) hydroxy-4-phenyl-3(S)-[[N-(2-quinolylcarbonyl)-L-asparaginyl]amino]butyl]-(4aS,8aS)-isoquinoline-3(S)-carboxamide methanesulfonate [illegible] and a molecular weight of 766.96. [illegible] saquinavir mesylate has the following structural formula:



$\cdot CH_3SO_3H$

Saquinavir mesylate is a white [illegible] with an aqueous solubility of 2.22 mg/mL at 25°C.

CLINICAL PHARMACOLOGY: Mechanism of Action: HIV protease cleaves viral polyprotein precursors to generate functional proteins [illegible] cleavage of viral polyprotein precursors is essential for maturation of infectious virus. Saquinavir [illegible] referred to as saquinavir is a synthetic peptide-like substrate analogue that inhibits the activity of HIV protease and prevents the cleavage of viral polyproteins.

Microbiology: Antiviral Activity [illegible] saquinavir was assessed in [illegible] and lymphocytes. Saquinavir inhibited [illegible] values (50% inhibitory concentration) [illegible] demonstrated additive to synergistic [illegible] with reverse transcriptase inhibitors [illegible] enhanced cytotoxicity.

[illegible] saquinavir have been selected in vitro [illegible] in the HIV protease at amino acid positions 48 [illegible] and [illegible] methionine.

Phenotypic and genotypic changes [illegible] from patients treated with saquinavir were also monitored in Phase I/II clinical trials. Phenotypic changes were defined as a [illegible] fold decrease in sensitivity [illegible] mutations [illegible] and/or G48V, the former predominating) [illegible] the incidence across studies of phenotypic and genotypic changes in the subsets of patients [illegible] for a period of 16 to 74 weeks [illegible] However, the clinical relevance of phenotypic and genotypic changes [illegible] with saquinavir therapy has not been established.

Table 1: Frequency of Genotypic and Phenotypic Changes in Selected Patients Treated with Saquinavir

	Genotypic	Phenotypic
[illegible]	[illegible]	5/11 (45%)
[illegible]	[illegible]	11/29 (38%)

[illegible] patients receiving monotherapy [illegible]

Cross-resistance [illegible] cross resistance between protease inhibitors [illegible] what effect saquinavir therapy [illegible] cross resistance between saquinavir and [illegible] different enzyme targets involved [illegible] saquinavir in vitro.

Pharmacokinetics: [illegible] properties of saquinavir have been evaluated in healthy volunteers [illegible] single and multiple oral doses of 25, 75, [illegible] and [illegible] doses of 6, 12, 36 or 72 mg (n=21).

[illegible] multiple dosing (600 mg tid) in HIV-infected [illegible] the plasma concentration versus time curve (AUC) was [illegible] after a single dose. HIV-infected patients administered saquinavir [illegible] same saquinavir after a meal or substantial [illegible] values which were about twice [illegible] the same treatment regimen (Table 2).

Table 2: Mean [illegible] AUC and Cmax in Patients and Healthy Volunteers

	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	40 [illegible] 44
[illegible]	[illegible]	[illegible]	[illegible] 96

[illegible] in 8 healthy volunteers who [illegible] saquinavir following a high fat breakfast (48 g protein [illegible] the bioavailability is thought to be due to a combination [illegible] first pass metabolism.

[illegible] in healthy volunteers [illegible]

[illegible] Adults: [illegible] 30% [illegible] saquinavir was approximately [illegible] range of 15 to 700 ng/mL [illegible] patients receiving saquinavir [illegible] concentrations were negligible when compared to concentrations [illegible]

Metabolism and Elimination in Adults: In vitro studies [illegible] that the metabolism of saquinavir is cytochrome P450 [illegible] CYP3A4 responsible for more than 90% of the hepatic [illegible] rapidly metabolized [illegible] 600 mg [illegible] was recovered in feces and urine [illegible] 4 subjects administered 10.5 mg of saquinavir [illegible] administered radioactivity was recovered in feces and [illegible] dosing. In mass balance studies, 13% of circulating [illegible] unchanged drug after oral administration and the remainder [illegible] following intravenous administration, 66% of circulating [illegible] drug and the remainder attributed to saquinavir metabolites [illegible] extensive first pass metabolism.

Systemic clearance of saquinavir was rapid, 1.14 L/h/kg [illegible] and 72 mg. The mean residence time of saquinavir was [illegible]

Special Populations: Hepatic or Renal Impairment: Saquinavir [illegible] hepatic or renal insufficiency has not been investigated [illegible]

Gender, Race and Age: Pharmacokinetic data were available [illegible] Pooled data did not reveal an apparent effect of gender or [illegible]

The effect of race on the pharmacokinetics of saquinavir [illegible] numbers of minorities for whom pharmacokinetic data were [illegible]

Saquinavir pharmacokinetics has not been investigated [illegible] patients < 16 years.

Drug Interactions: HIVID and ZDV: Concomitant use of INVIRASE [illegible] ZDV has been studied as triple combination [illegible] adults [illegible] absorption, metabolism and elimination of each of these [illegible] together.

Ketoconazole: Concomitant administration of ketoconazole [illegible] tid) to 12 healthy volunteers resulted in steady-state saquinavir [illegible] three times those seen with saquinavir alone. No dose [illegible] are coadministered at the doses studied. Ketoconazole [illegible] ministration with saquinavir.

Rifampin: Coadministration of rifampin (600 mg [illegible]) and [illegible] unteers decreased the steady-state AUC and Cmax of saquinavir [illegible]

Rifabutin: Preliminary data from 12 HIV-infected patients [illegible] saquinavir (600 mg tid) was decreased by 40% when [illegible] (300 mg qd).

INDICATIONS AND USAGE: INVIRASE in combination with [illegible] treatment of advanced HIV infection in selected patients [illegible] This indication is based on changes in surrogate markers [illegible] comitantly with either ZDV (in previously untreated patients [illegible] with prolonged zidovudine therapy). At present, there are [illegible] the activity of INVIRASE in combination with nucleoside [illegible] are also no results available from clinical trials containing [illegible] with INVIRASE on HIV disease progression or survival.

Description of Clinical Studies: The activity of INVIRASE [illegible] HIV infection has been evaluated in three double blind [illegible] with advanced HIV infection.

Advanced Patients without Prior ZDV Therapy: A dose [illegible] ZDV-naive patients (mean baseline CD4 [illegible] 179) studied INVIRASE [illegible] mg tid in combination with ZDV 200 mg tid compared to [illegible] In analyses of average CD4 changes over 16 weeks [illegible] mg tid + ZDV produced greater CD4 cell increases than [illegible] changes of ZDV in combination with doses of INVIRASE [illegible] that of ZDV alone.

Advanced Patients with Prior ZDV Therapy: In ACTG 229 [illegible] CD4 (165) with prolonged ZDV treatment (median 713 [illegible] INVIRASE 600 mg tid + HIVID + ZDV (triple combination [illegible] ZDV. In analyses of average CD4 changes over 24 weeks [illegible] increases in CD4 cell counts (see Fig. 2) compared to the [illegible] differences in CD4 changes among patients receiving INVIRASE [illegible]

Study NV14256 (North America) is an ongoing [illegible] INVIRASE 600 mg tid + HIVID to HIVID monotherapy [illegible] advanced HIV infection and at least 16 weeks of prior [illegible] with respect to clinical endpoints of disease progression [illegible] 16 weeks were conducted for a cohort of 423 patients [illegible] tion of INVIRASE + HIVID was associated with greater C[illegible] as monotherapy (see Fig. 3).

Comparisons of data across studies (NV14256 compared [illegible] INVIRASE was added to a regimen of prolonged prior [illegible] by continuing ZDV.

HIV RNA: The clinical significance of changes in HIV RNA [illegible] At present, this laboratory measure is available [illegible] in clinical trials. [illegible] 3 compares [illegible] RNA reductions [illegible] treatment arms in three clinical trials. Monotherapy arm [illegible] reductions were greater in INVIRASE/nucleoside combination [illegible] monotherapy controls.

CONTRAINDICATIONS: INVIRASE is contraindicated in patients [illegible] tivity to saquinavir or to any of the components contained [illegible]

PRECAUTIONS: *General:* The safety profile of INVIRASE [illegible] been established.

If a serious or severe toxicity occurs during treatment with [illegible] ed until the etiology of the event is identified or the toxicity [illegible] treatment with full dose INVIRASE may be considered [illegible] tion with INVIRASE, physicians should refer to the complete [illegible] dose adjustment recommendations and for information [illegible]

n Enrolled	
Prior ZDV	
n	
Median Duration (days)	
Log₁₀ Plasma RNA by PCR (copies/mL)	

DESCRIPTION

CLINICAL PHARMACOLOGY

Microbiology

Pharmacokinetics

INDICATIONS AND USAGE

Description of Clinical Studies

CONTRAINDICATIONS

PRECAUTIONS

Table 1 Frequency of Genotypic and Phenotypic Changes in Selected Patients

Table 2

Table 3

quinavir mesylate)

lume of distribution following intravenous admin- 700 L (CV 39%), suggesting saquinavir partitions % bound to plasma proteins over a concentration saquinavir 600 mg t.i.d. cerebrospinal fluid concentrations from matching plasma samples.

tudies using human liver microsomes have shown mal P450 medi- the specific isoenzyme the e hepatic me- ed on in vitro kinetics, ipond and the ive concentration of a ve (Ki) a clearly above achieved esage of dosing at 600 ran- % of the administered lec within 48 hours of ulat- asma was attributed to he parent saquinavir. Negligible recirculating radioactivity was attributed to the hydrophilic metabolites, suggesting that saquinavir undergoes

L/h/kg (CV 12%) after intravenous doses of 6 .3 hr was 7 hours (n=6).

ent. Saquinavir pharmacokinetics in patients with egulated (see PRECAUTIONS).

re available for 12 women in the studies, gender on the pharmacokinetics of saquinavir.

aquinavir has not been evaluated, but in the limited data were available.

ingested in patients >65 years of age in the studies

use of INVIRASE with HIV[2] nucleoside RTI and in adults. Pharmacokinetic data suggest that the of these drugs are unchanged when they are used

rtocnazole 200 mg q.d. and saquinavir 400 mg state saquinavir AUC and C_{max} values were 0 dose adjustment is required when the two drugs mazole pharmacokinetics were unaffected by coad-

q.d. and saquinavir 600 mg t.i.d. in 12 healthy vo- as of saquinavir by approximately 80%.

ted patients indicate that the steady state AUC of when saquinavir was coadministered with ritonavir

ation with nucleoside analogues is indicated for the patients (see Description of Clinical Studies). e numbers of patients who received INVIRASE in ed patients or HIV[2] in patients previously treated there are no reports available from randomized nucleoside analogues other than AZT in HIV[2] infected patients, the clinical benefit of combination therapy has been

INVIRASE in combination with AZT and in AZT- in three randomized trials in a total of 493 patients

dose ranging study (NV13330) where 90 patients ceived INVIRASE as doses of 200 mg t.i.d., 400 mg t.i.d. or 600 mg t.i.d. alone and 120 patients received AZT monotherapy. The CD4 cell response with the combination of INVIRASE 600 mg t.i.d. and AZT was significantly greater than the response with AZT monotherapy. The CD4 cell response with INVIRASE doses lower than 600 mg t.i.d. were not greater than

ACTG229/NV14255, 291 patients with mean baseline CD4 cell counts of 300 cells/mm³ were randomized to receive either INVIRASE 600 mg t.i.d. + AZT or HIVID 0.75 mg t.i.d. + AZT. After 24 weeks, the regimen containing INVIRASE produced greater CD4 cell increases than the regimen containing HIVID. There were no significant differences between the two regimens in the incidence of clinical progression or survival. In the INVIRASE + AZT arm, 4% of patients developed AIDS or died, while in the HIVID + AZT arm, 7% developed AIDS or died.

NV14256, a randomized, double-blind study comparing the safety and efficacy of INVIRASE monotherapy in patients with CD4 cell counts between 50 and 300 cells/mm³ and who had received no more than 6 months of prior AZT therapy, showed that INVIRASE 600 mg t.i.d. was superior to AZT monotherapy in delaying disease progression (i.e., development of AIDS or death). Patients receiving INVIRASE had a 51% reduction in the risk of developing AIDS or dying compared to patients receiving AZT. The median time to AIDS or death was 24 weeks for patients receiving INVIRASE and 12 weeks for patients receiving AZT.

Although the results of ACTG229/NV14255 suggest that when given in combination with AZT, INVIRASE 600 mg t.i.d. is more effective than INVIRASE 400 mg t.i.d., the results of NV14256 suggest that when given as monotherapy, INVIRASE 400 mg t.i.d. is more effective than INVIRASE 600 mg t.i.d. Therefore, the optimal dose of INVIRASE when given in combination with AZT has not been established.

600 or patients with creatinine clearance greater than 50 ml/min. No dose adjustment is necessary for patients with creatinine clearance greater than 50 ml/min. The safety and efficacy of INVIRASE in patients with creatinine clearance less than 50 ml/min have not been established.

Studies with INVIRASE in combination with other antiretroviral agents have not been conducted. Because of the potential for overlapping toxicities, caution should be exercised when administering INVIRASE with other drugs that are known to cause peripheral neuropathy, pancreatitis, hepatic dysfunction, hyperlipidemia, and/or rash.

INVIRASE™ (saquinavir mesylate)

Fig. 1 Mean CD_4 Changes (cells/mm³) from Baseline in Study V13330 (Italy)

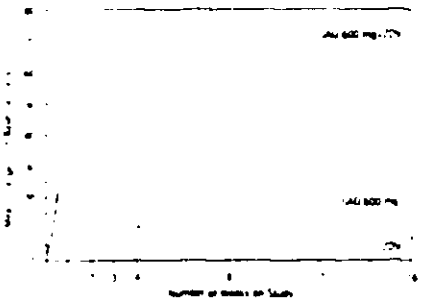


Fig. 2 Mean CD_4 Changes (cells/mm³) from Baseline in Study ACTG229/NV14255

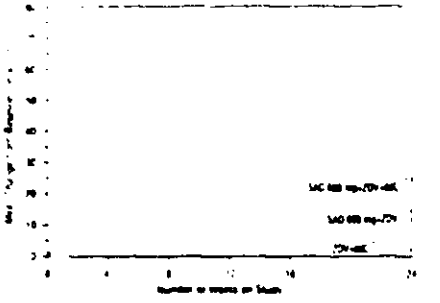


Fig. 3 Mean CD_4 Changes (cells/mm³) from Baseline in Study NV14256

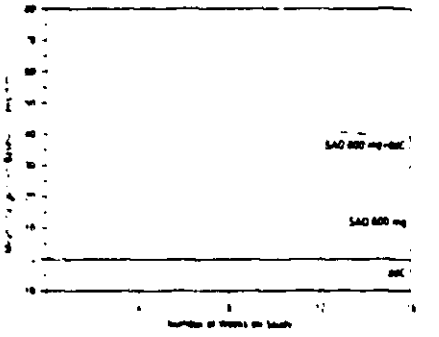


Table 3 Summary of Week 24 Plasma RNA Results from Major INVIRASE Clinical Studies*

	NV14255 ACTG229 (USA)				Surrogate Marker Analysis NV14256 (North America)		
	ZDV-experienced						
	ddC	ZDV + ddC	ZDV + SAQ	ZDV + ddC + SAQ	ddC	SAQ	SAQ + ddC
	90	90	90	98	145	159	147
	0%	4%	4%	0%	134	151	136

...ine plus zidovudine (ZDV) and ...

...DV and saquinavir 1600 mg tid in HIV-negative ...
...ed by administration ...

...ted patients indicate that the ... when saquinavir was coadministered with ritonavir ...

...ation with nucleoside analogues is indicated for the ... patients. Discontinuation of therapy in studies between ... markers in patients who initiated the INVIRASE ... ed patients or HIV/O (in patients previously treated ... there are no results available from ... nucleoside analogues other than ZDV ... continuing the clinical benefit of combination therapy ... dving.

INVIRASE in combination with HIVID and no ZDV ...), blind, randomized trials in a total of 870 patients ...

...ose ranging study (study NV14230) ... enrolled in 9... ...oaded INVIRASE at doses of 75 mg, 200 mg and 600 ...oaded to INVIRASE 600 mg tid alone and ZDV alone ...es. Treatment with the combination of INVIRASE 600 ...ases than ZDV monotherapy (see Fig. 1). The CD4 ...VIRASE lower than 600 mg tid were not greater than ...

...ACTG229/NV14255: 295 patients ... baseline ...(up 213 days) were randomized to receive either ...nbination: INVIRASE 600 mg tid + ZDV + HIVID ... 24 weeks. The triple combination produced greater ...ed to that of HIVID + ZDV. There were no significant ...ving INVIRASE + ZDV and HIVID + ZDV.

...ing randomized, double-blind study comparing ...erapy and INVIRASE monotherapy in patients with ...o prior ZDV treatment. The study remains blinded ...gression. However, analysis of CD4 changes ...patients. These analyses showed that the combina... ...greater CD4 increases than either agent in INVIRASE ...

...Compared to ACTG229/NV14255, suggests that when ... prior zidovudine there was little additional contributed ...

...HIV RNA measurements have not been established ... an experimental basis to measure antiviral activity ...uctions at 16 weeks among the RNA combination ...rapy arms are included for reference. Overall, RNA ...se combination regimens compared to nucleoside ...

...ded in patients with clinically significant hypersensitivity ...contained in the capsule.

...INVIRASE in pregnant women ... No adequate ... has not ...

...ment with INVIRASE. INVIRASE should be discontinued ...e the lowest ... recommended. Although the ... of ... ...observed, the ... ...amount of ... Until ... combina... ...the combined potential for the many drugs to ... ...ction ... ...drug ... ...adverse reactions ...

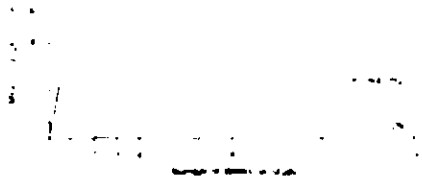


Fig. 2 Mean CD_4 Changes (cells/mm³) from Baseline in Study ACTG229/NV14255

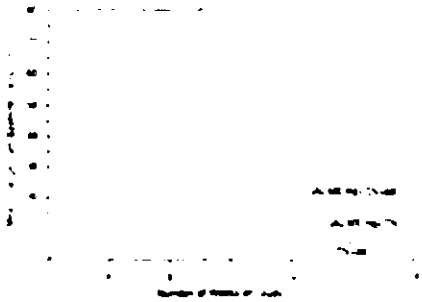


Fig. 3 Mean CD_4 Changes (cells/mm³) from Baseline in Study NV14256

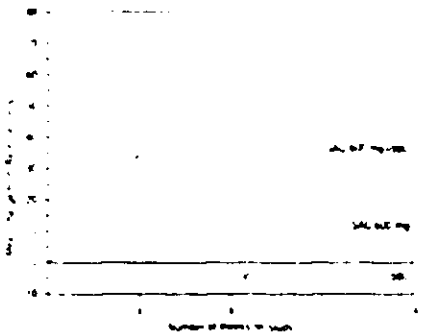


Table 3 Summary of Mean Log_{10} Plasma RNA Results from Major INVIRASE Clinical Studies*

	NV14255/ACTG229 (USA) ZDV-experienced			Surrogate Marker Analysis NV14256 (North America)		
	ZDV+ddC	ZDV+SAQ	ZDV+ddC+SAQ	ddC	SAQ	SAQ+ddC
	100	99	98	145	159	147
	99	98	97	134	151	136
	459	713	647	514	459	442
	98	97	96	114	124	119
	4.8 (96)	4.8 (96)	4.8 (96)	5.2 (114)	5.1 (124)	5.1 (119)
	-0.1	0.0	-0.5	-0.4	-0.1	-0.6
	-0.2	0.0	-0.5	-	-	-

...saquinavir (SAQ) at 600 mg tid - indicates not applicable

INVIRASE™

XXXXXXXX

INVIRASE™ (saquinavir mesylate)

...solution should be taken with other antiretroviral agents. ... patients with hepatic insufficiency since patients ... Child-Pugh Class B ... those with abnormal liver function were not included in clinical studies.

Resistance Cross resistance ... Cross resistance between protease inhibitors has not been fully evaluated. ... the extent to which prior exposure to saquinavir therapy will have on the activity of subsequent protease inhibitor ... therapy.

Information for Patients ... patients should be informed that INVIRASE ... a cure for HIV infection and that they may ... opportunistic infections ... advanced HIV infection, including ... Patients should be advised that ... the transmission of HIV, sexual exposure, and patients should be advised to ... while using INVIRASE.

Patients should be ... that INVIRASE ... They should be informed that ... INVIRASE ... reduce the risk of transmitting HIV to others through sexual contact or blood contamination.

Patients should be ... that INVIRASE should be given within 2 hours after a full meal (see *Pharmacokinetics*). When INVIRASE is given ... concentrations of saquinavir in the blood are substantially ... and may result in ... failure.

Laboratory tests ... Certain abnormalities in ... laboratory tests have been associated with the use of INVIRASE ... These tests should be performed prior to initiating INVIRASE therapy and at appropriate ... to provide comprehensive information concerning laboratory test alterations associated with the use of individual ... protease inhibitors, physicians should refer to the complete product information for these drugs.

Drug Interactions *Rifampin* ... INVIRASE should not be administered concomitantly with rifampin since rifampin ... saquinavir ... concentrations by 80% (see *Pharmacokinetics*). Rifabutin also substantially ... saquinavir ... concentrations by 40%. Other drugs that induce CYP3A4 (eg, ... phenobarbital, ... carbamazepine) may also reduce saquinavir plasma concentrations ... Because these drugs are warranted, physicians should consider using alternative ... in patients receiving INVIRASE.

Other Potentially Interactive Medications ... Coadministration of saquinavir with drugs that are known to be potent inhibitors ... may increase saquinavir concentrations, which may in turn prolong QT interval ... Such drugs ... should not be used with INVIRASE ... Although INVIRASE is not a ... inhibitor ... drug interaction studies with INVIRASE and terfenadine ... should use alternatives to terfenadine or astemizole when ... INVIRASE ... Other compounds that are substrates ... CYP3A4 ... may ... saquinavir ... dapsone, quinidine, trazodone, and clarithromycin. Other compounds that are ... metabolized by CYP3A4 ... may have elevated plasma ... when coadministered with INVIRASE; therefore, patients should be monitored ... and dose ... as ... [illegible]

Carcinogenesis, Mutagenesis and Impairment of Fertility *Carcinogenesis* Carcinogenicity studies in rats and mice have not ... been completed.

Mutagenesis Saquinavir ... and demonstrated ... with and without metabolic activation where appropriate have shown that saquinavir has no mutagenic activity in vitro in either bacterial (Ames test) or mammalian (eg, ... Chinese hamster lung ... HPRT test). Saquinavir does not induce chromosome damage ... in the mouse micronucleus assay or in vitro in human peripheral blood lymphocytes, and does not induce primary DNA damage in vitro in the unscheduled DNA synthesis test.

Impairment of Fertility ... Fertility and reproductive performance were not affected in rats at plasma saquinavir ... levels ... at or ... those achieved in humans at the recommended dose.

Pregnancy *Teratogenic Effects* ... Pregnancy ... and reproduction studies conducted with saquinavir in rats have shown ... and embryotoxicity at ... plasma exposures (AUC values) up to five times ... those achieved at the recommended dose in humans. In rabbits at plasma exposures four times ... those achieved ... dose, ... abortions ... in rats indicated that exposure to saquinavir ... during ... through ... at plasma concentrations (AUC values) up to five times ... those achieved ... dose had no effect on the survival, growth and ... Animal reproduction studies are not always predictive ... Saquinavir ... should be used during pregnancy only if ... and only after ... the potential ... to the fetus. ... reports of infants ... born after ... exposure ... during ... pregnancy ... congenital anomalies ...

Nursing Mothers ... Saquinavir ... is excreted in human milk. Because many drugs are excreted ... and because ... serious adverse reactions in nursing infants, ... a decision ... to discontinue nursing or discontinue the drug, ... the importance of the drug to the mother.

Pediatric Use ... Safety and effectiveness ... in infected children or adolescents younger than ... years ... have not been established.

ADVERSE REACTIONS ... The safety of INVIRASE was studied in 688 patients who received ... INVIRASE ... ZDV and ... ddC). The most frequently reported adverse events ... were ... which ... to be associated with ZDV and ddC, ... abdominal discomfort and nausea.

INVIRASE ... has been ... associated with ... major toxicities ... See the complete product information for these ... drugs ... appropriate ... drug associated adverse reactions to other ...

... patients receiving INVIRASE 600 mg ... Median duration of treatment in ... was ... weeks; median duration of treatment among ... was 47 weeks.

... have been reported during clinical ... of INVIRASE ... related to use of study drugs: confusion, ... attempted suicide, Stevens ... In ... associated with increased liver function ... headache and thrombocytopenia ...

Table 4 Percentage of Patients, by Study ... Relationship and of Moderate ...

ADVERSE EVENT	
GASTROINTESTINAL	
Diarrhea	
Abdominal Discomfort	
Nausea	
Dyspepsia	
Abdominal Pain	
Mucosal Damage	
Buccal Mucosal Ulceration	
CENTRAL AND PERIPHERAL NERVOUS SYSTEM	
Headache	
Paresthesia	
Extremity Numbness	
Dizziness	
Peripheral Neuropathy	
BODY AS A WHOLE	
Asthenia	
Appetite Disturbances	
SKIN AND APPENDAGES	
Rash	
Pruritus	
MUSCULOSKELETAL DISORDERS	
Musculoskeletal Pain	
Myalgia	

- indicates no events reported

Table 5 Percentage of Patients, by ...

BIOCHEMISTRY	
Calcium (high)	
Creatine Phosphokinase	
Glucose (low)	
Glucose (high)	
Phosphorus	
Potassium (high)	
Potassium (low)	
Serum Amylase	
SGOT (AST)	
SGPT (ALT)	
Total Bilirubin	
Uric Acid	
HEMATOLOGY	
Neutrophils (low)	
Hemoglobin (low)	
Platelets (low)	

*Marked Laboratory Abnormality defined as a shi...

Monotherapy and Combination Studies Other cl... remotely related to INVIRASE, including those in ... studies NV14255/ACTG229 and NV14256, and t... body system.

Body as a Whole Allergic reaction, chest pain ... retrosternal pain, shivering, wasting syndrome, we...

Cardiovascular Cyanosis, heart murmur, heart va... vein distended

Endocrine/Metabolic Dehydration, dry eye syndro...

Gastrointestinal Cheilitis, constipation, dyspnagia, ... gastralgia, gastritis, gastrointestinal inflammation, ... rhoids, hepatomegaly, hepatosplenomegaly, mele..., ... palmed disorder, salivary gland disorder, stomatiti...

Hemic/Lymphatic Anemia, micronormocytic, pancytop...

Musculoskeletal Arthralgia, arthritis, back pain, c... ness, tissue changes, trauma

Neurological Ataxia, bowel movements frequent, cor... rate disorder, hyperesthesia, hypertelia, hypertelia, ... polyneuritis, progressive multifocal leukoencephalo...

Psychological Agitation, amnesia, anxiety, depres..., insomnia, intellectual ability reduced, irritability, ... disorder, somnolence, speech disorder

Reproductive System Prostate enlarged, vaginal dis...

Resistance Mechanism Abscess, angina, tonsillar, ... zoster infection, bacterial infection, mycotic infect..., tumor

Respiratory Bronchitis, cough, dyspnea, epistaxis, ... respiratory disorder, rhinitis, sinusitis, upper respira...

Skin and Appendages Acne, dermatitis, dermatitis, ... cutosis, hair changes, hot flushes, photosensitiv..., ... lobadular skin disorder, skin nodule, skin ulcer, ... xeroderma

Special Senses Blepharitis, earache, ear pressure, e... ation, tinnitus, visual disturbance

Urinary System Micturition disorder, urinary tract ...

OVERDOSAGE No acute toxicities or sequelae we... INVIRASE as a single dose. The patient was treate...

Information for [illegible] and that [illegible] opportunistic [illegible] illnesses, and [illegible] the [illegible] of a physician while using INVIRASE. Patients [illegible] be informed that [illegible] others through [illegible].

Patients should be advised that INVIRASE should be taken within 2 hours after a full meal (see *Pharmacokinetics*). [illegible] INVIRASE [illegible] concentrations of saquinavir in the blood are substantially reduced and may [illegible].

Laboratory Tests [illegible] have been associated with the use of INVIRASE [illegible] prior to initiating INVIRASE therapy and at appropriate [illegible] alterations [illegible] complete product [illegible].

Drug Interactions [illegible] INVIRASE should not be administered concomitantly with rifampin, since [illegible] by 80% (see *Pharmacokinetics*). Rifabutin also substantially reduces saquinavir plasma concentrations by 40%. Other drugs that induce CYP3A4 (e.g., phenobarbital, phenytoin, dexamethasone, carbamazepine) may also reduce saquinavir plasma concentrations. [illegible] is warranted, physicians should consider using alternatives when a patient is taking INVIRASE.

Other Potential Interactions Coadministration of terfenadine or astemizole with drugs that are known to be potent inhibitors of the cytochrome P450 3A pathway (e.g., ketoconazole, itraconazole, etc.) may lead to elevated plasma concentrations of terfenadine or astemizole, which may in turn prolong QT intervals leading to rare cases of serious cardiovascular adverse events. Although INVIRASE is not a strong inhibitor of cytochrome P450 3A [illegible] studies with INVIRASE and terfenadine or astemizole have not been [illegible]. Physicians should use alternatives to terfenadine or astemizole when [illegible] INVIRASE [illegible] antihistamines. Other compounds that are substrates of CYP3A4 [illegible] clindamycin, dapsone, quinidine, triazolam) may have elevated plasma concentrations when coadministered with INVIRASE; therefore patients should be monitored for [illegible].

Carcinogenesis, Mutagenesis and Impairment of Fertility Carcinogenesis: Carcinogenicity studies in rats and mice [illegible].

Mutagenesis [illegible] with and without metabolic activation where appropriate [illegible] in either bacterial (Ames test) or mammalian [illegible] (HGPRT) test. Saquinavir does not induce chromosomal [illegible] in vitro in human peripheral blood lymphocytes [illegible] DNA damage [illegible] in the unscheduled DNA synthesis test.

Impairment of fertility and reproductive performance were not affected in rats at plasma exposures (AUC [illegible]) those achieved in humans at the recommended dose.

Pregnancy [illegible] reproduction studies conducted with saquinavir in rats have shown [illegible] plasma exposures (AUC values) up to five times those achieved [illegible] plasma exposures four times those achieved [illegible] studies in rats indicated that exposure to saquinavir [illegible] at plasma concentrations (AUC values) up to five times those achieved in humans at the recommended dose had no effect on the survival, growth and development of offspring [illegible] because animal reproduction studies are not always predictive of human response, INVIRASE should be used during pregnancy after taking into account the importance of the drug [illegible] there are no reports of infants being born after women receiving INVIRASE [illegible].

Nursing Mothers [illegible] whether INVIRASE is excreted in human milk. Because many drugs are excreted [illegible] the potential for serious adverse reactions in nursing infants [illegible] whether to discontinue nursing or discontinue the drug [illegible] INVIRASE to the mother.

Pediatric Use [illegible] INVIRASE in HIV-infected children or adolescents younger than [illegible].

ADVERSE REACTIONS [illegible] The safety of INVIRASE was studied in 688 patients [illegible] combination with ZDV and/or HIVID (zalcitabine, ddC). The majority of [illegible]. The most frequently reported adverse events among patients [illegible] INVIRASE [illegible] those toxicities known to be associated with ZDV and HIVID [illegible] diarrhea, abdominal discomfort and nausea.

INVIRASE [illegible] frequency or severity of known major toxicities associated with the use of [illegible] refer to the complete product information for these drugs [illegible] appropriate) for drug associated adverse reactions to other nucleoside analogues.

Table 4 lists [illegible] adverse events that occurred in ≥2% of patients receiving INVIRASE 600 mg tid alone or in combination with ZDV and/or HIVID in two trials. Median duration of treatment in NV14255/ACTG229 [illegible] combination study was 48 weeks; median duration of treatment among the [illegible] for safety (n=451) in NV14256 was 42 weeks.

Rare [illegible] the following serious adverse experiences have been reported during clinical trials of INVIRASE and were considered at least possibly related to use of study drugs: confusion [illegible] hemolytic anemia, attempted suicide, Stevens-Johnson syndrome [illegible] cutaneous reaction associated with increased liver function tests [illegible] thrombophlebitis, headache and thrombocytopenia, exacerbation of [illegible] elevated liver function tests, jaundice, ascites, and right [illegible].

Table 5 [illegible] with marked laboratory abnormalities in studies [illegible]. Marked laboratory abnormalities are defined as a Grade 3 or 4 [illegible] or a Grade 4 abnormality in a patient with a [illegible].

Musculoskeletal [illegible]

[illegible]

Myalgia

[illegible] no events reported

Table 5 Percentage of Patien[illegible]

BIOCHEMISTRY	
Calcium (high)	
Creatine Phosphokinase	
Glucose (low)	
Glucose (high)	
Phosphorus	
Potassium (high)	
Potassium (low)	
Serum Amylase	
SGOT (AST)	
SGPT (ALT)	
Total Bilirubin	
Uric Acid	
HEMATOLOGY	
Neutrophils (low)	
Hemoglobin (low)	
Platelets (low)	

[a] Marked Laboratory Abnormality defined [illegible]

Monotherapy and Combination Studies [illegible] remotely related to INVIRASE, including [illegible] studies NV14255/ACTG229 and NV142[illegible] body system.

Body as a Whole Allergic reaction, [illegible] retrosternal pain, shivering, wasting syn[illegible]

Cardiovascular Cyanosis, heart murmu[illegible] vein distended

Endocrine/Metabolic Dehydration, dry e[illegible]

Gastrointestinal Cheilitis, constipation, [illegible] gastralgia, gastritis, gastrointestinal infla[illegible] rhoids, hepatomegaly, hepatosplenome[illegible] parotid disorder, salivary glands disorder[illegible]

Hematologic Anemia, microhemorrhage[illegible]

Musculoskeletal Arthralgia, arthritis, [illegible] ness, tissue changes, trauma

Neurological Ataxia, bowel movements [illegible] rate disorder, hyperesthesia, hyperreflexia [illegible] poliomyelitis, progressive multifocal leu[illegible]

Psychological Agitation, amnesia, anx[illegible] insomnia, intellectual ability reduced [illegible] disorder, somnolence, speech disorder[illegible]

Reproductive System Prostate enlarge[illegible]

Resistance Mechanism Abscess, ang[illegible] zoster, infection bacterial, infection my[illegible] tumor

Respiratory Bronchitis, cough, dyspne[illegible] respiratory disorder, rhinitis, sinusitis, [illegible]

Skin and Appendages Acne, dermatitis [illegible] culosis, hair changes, hot flushes, [illegible] lopapular, skin disorder, skin nodule [illegible] xeroderma

Special Senses Blepharitis, earache, ea[illegible] ation, tinnitus, visual disturbance

Urinary System Micturition disorder, u[illegible]

OVERDOSAGE No acute toxicities or [illegible] INVIRASE as a single dose. The patie[illegible] after ingestion. In an exploratory Ph[illegible] (1200 mg q4h) there were no serious [illegible]

DOSAGE AND ADMINISTRATION Th[illegible] nucleoside analogue is three 200-mg [illegible] meal. The recommended doses of HI[illegible] are: HIVID 0.75 mg three times daily [illegible]

[redacted]

INVIRASE™ (saquinavir mesylate)

[illegible] with Clinical Adverse Experiences Considered at Least Possibly Related to Study Drug or of Unknown or Life-threatening Intensity, Occurring in ≥2% of Patients in NV14255/ACTG229 and NV14256

	NV14255/ACTG229			NV14256		
	SAQ+ZDV n=99	SAQ+ddC+ZDV n=98	ddC+ZDV n=100	ddC n=145	SAQ n=159	SAQ+ddC n=[illegible]
	3 0	1 0	-	1 4	3 8	3 4
	2 0	3 1	4 0	1 4	[illegible]	[illegible]
	-	3 1	3 0	0 7	1 9	[illegible]
	1 0	1 0	2 0	2 1	-	0 [illegible]
	2 0	1 0	2 0	0 7	1 9	0 [illegible]
	-	-	4 0	1 4	-	0 [illegible]
	-	2 0	2 0	9 0	2 5	4 [illegible]
	2 0	2 0	2 0	4 1	0 6	0 [illegible]
	2 0	3 1	4 0	0 7	1 0	1 0
	2 0	1 0	4 0	-	-	0 7
	-	2 0	1 0	-	-	-
	-	1 0	2 0	5 5		4 8
	6 1	9 2	10 0	0 7	1 3	[illegible]
	-	1 0	2 0	-		
	-	-	3 0	0 7	1 3	1 4
	-	-	2 0	-		
	2 0	2 0	4 0	-	0 6	0 [illegible]
	1 0		3 0	1 4		

[illegible] Treatment Group with Marked Laboratory Abnormalities* in NV14255/ACTG229 and NV14256

	NV14255/ACTG229			NV14256		
	SAQ+ZDV n=99	SAQ+ZDV+ddC n=98	ZDV+ddC n=100	ddC n=145	SAQ n=[illegible]	SAQ+ddC n=[illegible]
	[illegible]	[illegible]	[illegible]	<1	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	6	4	
			[illegible]	4	[illegible]	[illegible]
			[illegible]	0		
	[illegible]			0		[illegible]
	[illegible]		[illegible]	0	[illegible]	[illegible]
	[illegible]			<1		[illegible]
				3		
				3		
				0		
	[illegible]		[illegible]	Not assessed	Not assessed	Not assessed
	[illegible]	[illegible]	3	0		
		[illegible]	[illegible]	0	[illegible]	[illegible]
			[illegible]	0		

* From Grade 0 to at least Grade 3 or from Grade 1 to Grade 4 (ACTG Grading [illegible])

[illegible] adverse experiences of any intensity [illegible] ≥2% of patients on arms containing INVIRASE [illegible] those in smaller clinical trials, are listed below [illegible]

[illegible] edema, fever, [illegible] [illegible] [illegible] weight decrease

[illegible] disorder, hyper[illegible], hypotension, syncope

[illegible] hyperglycemia, weight increase, [illegible]

[illegible] [illegible] [illegible] [illegible] [illegible] discolored [illegible] [illegible] hemorrhage [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] vomiting

[illegible] splenomegaly, thrombocytopenia

[illegible] cramps, muscle, musculoskeletal disorders, [illegible]

[illegible] [illegible] [illegible] face pain, facial [illegible] [illegible]

[illegible] dreaming, excessive, euphoria, hallucination [illegible] [illegible] disorder [illegible] [illegible]

Monitoring of Patients: [illegible] INVIRASE therapy and at appropriate [illegible] recommendations for other nucleoside analogues [illegible] information for these drugs.

Dose Adjustment for [illegible] INVIRASE [illegible] may be associated with INVIRASE [illegible] 600 mg tid are not recommended [illegible] of combination therapy with INVIRASE [illegible] nucleoside analogue should be based on the [illegible] Physicians should refer to the complete product information for these drugs [illegible] recommendations and drug associated adverse [illegible]

HOW SUPPLIED: INVIRASE 200 mg [illegible] opaque capsules with ROCHE and 0245 [illegible] NDC 0004-0245-15 [illegible]

The capsules should be stored at [illegible] tightly closed bottles.

SAQ+ZDV n=99	SAQ+ZDV+ddC n=98	ZDV+ddC n=100	ddC n=145	SAC n=159	[illegible]
3.0	1.0		1.4	3.8	3.4
2.0	3.1	4.0	1.4	1.3	[illegible]
-	3.1	3.0	0.7	1.9	[illegible]
1.0	1.0	2.0	[illegible]		[illegible]
2.0	1.0	2.0	0.7	1.9	[illegible]
-	-	4.0	1.4	-	
-	2.0	2.0	9.0	[illegible]	[illegible]
2.0	2.0	2.0	4.1	0.6	[illegible]
2.0	3.1	4.0	0.7	1.0	[illegible]
2.0	1.0	4.0	-		[illegible]
-	2.0	1.0	-		
-	1.0	2.0	5.5	-	4.8
6.1	9.2	10.0	0.7	1.3	[illegible]
-	1.0	2.0	-		
-	-	3.0	0.7	1.3	1.4
-	-	2.0	-		
2.0	2.0	4.0	-	0.6	
1.0	-	3.0	1.4	-	

by Treatment Group, with Marked Laboratory Abnormalities* in NV14255/ACTG229 and NV14256

NV14255/ACTG229			NV14256		
SAQ+ZDV n=99	SAQ+ZDV+ddC n=98	ZDV+ddC n=100	ddC n=145	SAC n=159	SAC+ddC n=[illegible]
1	0	0	<1	0	[illegible]
10	12	[illegible]	6	4	[illegible]
0	0	0	4	5	4
0	0	0	0	[illegible]	[illegible]
[illegible]	[illegible]	0	0	[illegible]	[illegible]
0	0	0	[illegible]	[illegible]	[illegible]
0	0	0	0	[illegible]	[illegible]
[illegible]	1	1	<1	[illegible]	[illegible]
[illegible]	2	0	3	[illegible]	[illegible]
0	3	1	[illegible]	[illegible]	[illegible]
[illegible]	0	[illegible]	0	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	Not assessed	Not assessed	Not assessed
[illegible]	[illegible]	[illegible]	0	[illegible]	
[illegible]	0	[illegible]	0	[illegible]	
0	[illegible]	2	0	[illegible]	

*[illegible] from Grade 0 to at least Grade 3 or from Grade 1 to Grade 4 (ACTG Grading System)

[illegible] clinical adverse experiences of any intensity [illegible] at least [illegible] in <2% of patients on arms containing INVIRASE in [illegible] those in smaller clinical trials, are listed below [illegible]

[illegible] weight decrease

[illegible] hypertension, hypotension [illegible]

[illegible] hyperglycemia, weight increase [illegible]

[illegible] vomiting

[illegible] splenomegaly, thrombocytopenia

[illegible] cramps muscle, musculoskeletal disorders [illegible]

[illegible] confusion, convulsions [illegible] numbness face [illegible] seizures, tremor

[illegible] depression, dreaming excessive, euphoria, hallucination [illegible] libido disorder, overdose effect [illegible]

[illegible] discharge

[illegible] candidiasis, hepatitis, herpes simplex, herpes [illegible] influenza, lymphadenopathy

[illegible] pharyngitis, pneumonia [illegible] respiratory tract infection

[illegible] eczema, erythema [illegible] pigment changes skin [illegible] sweating increased, urticaria [illegible]

[illegible] hearing decreased [illegible]

[illegible] tract infection

[illegible] a patient who ingested 8 grams of [illegible] with induction of emesis within 2 to 4 hours [illegible] dosing with INVIRASE at 7200 mg daily [illegible] through the first 25 weeks of treatment

[illegible] INVIRASE in combination with a [illegible] in combination therapy [illegible] three times daily as appropriate

Monitoring of Patients: Clinical chemistry tests should be performed prior to initiating INVIRASE therapy and at appropriate intervals thereafter. For comprehensive patient monitoring recommendations for other nucleoside analogues, physicians should refer to the complete product information for these drugs.

Dose Adjustment for Combination Therapy with INVIRASE: For toxicities that may be associated with INVIRASE, the drug should be interrupted. INVIRASE at doses less than 600 mg tid are not recommended since lower doses have not shown antiviral activity. For recipients of combination therapy with INVIRASE and nucleoside analogues, dose adjustment of the nucleoside analogue should be based on the known toxicity profile of the individual drug. Physicians should refer to the complete product information for these drugs for comprehensive dose adjustment recommendations and drug associated adverse reactions of nucleoside analogues.

HOW SUPPLIED: INVIRASE 200 mg capsules [illegible] light brown and green opaque capsules with ROCHE and 0245 imprinted [illegible] NDC 0004-0245-15.

The capsules should be stored at 59° to 86°F [illegible] in tightly closed bottles.

Manufactured by [illegible] La Roche [illegible]

[illegible]

Roche Laboratories

A Member of the Roche Group

[illegible]

[illegible]

13 06 [illegible]

13 29 [illegible]

Revised: December 1995

Printed in U.S.A.

DRAFT

Clinical and Statistical Review of NDA 20-628, INVIRASE™ (saquinavir)

1.0 General Information

Medical Officer:	Jeffrey S. Murray, M.D.
Statistician:	Kazem Kazempour, PhD.
Date Received:	Aug. 31, 1995
Review Completed:	Feb. 5, 1996
Sponsor:	Hoffman-La Roche Inc. 340 Kingsland Street Nutley, NJ 07110-1199
Drug:	Ro 31-8959/003, INVIRASE (Saquinavir)
Pharmacologic Category:	Anti-retroviral, HIV-1 protease inhibitor
Dosage Form and Routes of Administration:	200 mg hard gelatin capsules, oral
Proposed Indications:	1 INVIRASE in combination with nucleoside analogues for the treatment of advanced HIV infection. 2 INVIRASE as monotherapy for the treatment of advanced HIV infection in patients who are intolerant of currently available antiretrovirals.
Related NDAs:	None
Related Reviews:	None

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3.0 Materials Reviewed

NDA 20-628 was submitted in hard copy and as a computer assissted NDA (CANDA).

4.0 Chemistry/Manufacturing Controls

For a complete review of CMC, please refer to Dr. Paul Liu's review.

5.0 Animal Pharmacology/Toxicology

Please refer to Dr. K.M. Wu's review.

6.0 Microbiology

Please refer to Dr. Nara Batulla's review for a summary of preclinical virology and in vitro resistance testing. Also refer to Dr. Lauren Iaconos-Connor's review for issues regarding clinical saquinavir resistance and cross-resistance to other antiretroviral drugs.

7.0 Clinical Background

7.1 Relevant human experience

Roche submitted all of the relevant human experience with saquinavir in the NDA. The clinical activity closing date was Jan 1, 1995, the safety data closing date was June 1, 1995.

7.2 Human Pharmacology, Pharmacokinetics

Please refer to the reviews written by Drs. Janice Jenkins and Chandra Sahjawalla (Division of Biopharmaceutics) for human pharmacology issues. There were several significant clinical pharmacology issues including a marked food effect on the bioavailability of saquinavir, poor bioavailability of the current formulation, and important pharmacokinetic interactions with rifampin and rifabutin. These issues are appropriately addressed in the final product label.

7.3 Background information

Roche presented their Phase 3 clinical confirmatory program to a closed session of the Division of Antiviral Drug Products advisory committee on Jan., 1995. In general, committee members present were in agreement with the design of the two confirmatory trials. One criticism was the exclusion of patients with CD4 counts less than 50 cells/mm^3. Although this subgroup will not be specifically evaluated for efficacy in clinical trials, limited safety data is being collected from this subgroup within an expanded access program.

7.4 Description of Clinical Data Sources

In this review, except for direct quotes from the applicant Hoffmann-La Roche (Roche) or the label, INVIRASE™ (saquinavir, Ro 31-8959) will be referred to as SAQ. Table 1 lists the clinical studies included in the NDA. All of these studies were designed to collect safety and surrogate marker data. Two of these trials are ongoing. NV14256 remains blinded with respect to clinical endpoints and is part of Roche's accelerated approval commitments as a clinical confirmatory study. Surrogate marker data from a cohort of 451 patients from this study were analyzed and included in this NDA. Study EV14757 is a ongoing trial collecting surrogate marker data in patients receiving doses of SAQ greater than the proposed marketing dose of 600 mg tid (1800 mg/day). Some preliminary data from this study were submitted in an update to the NDA.

Table 1 SAQ Clinical trials evaluating activity/efficacy

PROTOCOL	DESIGN	TREATMENT ARMS	SUBJECTS
O-13328 *United Kingdom*	Phase 1/2, double blind *randomized* 12/91-9/92	SAQ 25 mg tid SAQ 75 mg tid SAQ 200 mg tid SAQ 600 mg tid	49 treatment naive
V-13329 France	Phase 1/2, randomized double-blind 12/91-9/92	SAQ 75 mg tid SAQ 200 mg tid SAQ 600 mg tid	61 ZDV experienced
V-13330 Italy	Phase 1/2, randomized double-blind 2/92-2/93	ZDV+SAQ 75 mg tid ZDV+SAQ 200 mg tid ZDV+SAQ 600 mg tid ZDV 200 mg tid SAQ 600 mg tid	94 treatment naive
ACTG 229/NV14255	Phase 2, randomized double blind 3/93-12/93	ZDV+ddC ZDV+SAQ 600 mg tid ZDV+SAQ+ddC	302 ZDV experienced
NV 14256 surrogate analysis (clinical ongoing)	Phase 3 randomized double blind surrogate and clinical endpoints	ddC SAQ 600 mg tid ddC+SAQ	451 ZDV experienced
EV14757 (ongoing)	Phase 1/2, open-label dose ranging	SAQ 800 mg q4h (3600 mg) SAQ 1200 mg q4h (7200 mg)	41 treatment naive

In addition to the studies listed in Table 1, blinded safety data is available from patients participating in additional ongoing clinical trials and "roll-over" protocols. For a summary of the extent of exposure and a listing of the number of patients included in the entire safety data base, refer to section 10.0, "Overview of Safety."

8.1 Indication # 1 Combination therapy

8.1.1 ACTG 229/NV14255

"A double-blind, randomized, phase 2 study of Ro-31-8959 plus zidovudine versus zidovudine plus zalcitabine versus Ro-31-8959 plus zidovudine plus zalcitabine."

For the remainder of the review this study will be referred to as ACTG 229.

8.1.1.1 Protocol

8.1.1.1.1 Objective/Rationale

The primary objectives of this study were to investigate the activity, safety and tolerance of the three different treatment arms. Primary indicators of activity were CD4 response and the reduction in viral load as measured by peripheral blood mononuclear cell (PBMC) virus culture.

The secondary objectives were

1. To determine the reduction in HIV viral burden as measured by plasma viraemia, p24 antigen, and the RNA polymerase chain reaction (PCR).
2. To investigate changes in other immunological tests: CD8, serum neopterin, β-2-microglobulin, CD4%, CD4/CD38/HLA-DR and CD8/CD38/HLA-DR activation markers.
3. To determine pharmacokinetic parameters, Cmax, tmax, and, AUC for the three drugs administered in different combinations and urinary excretion parameters for ZDV and ddC.
4. To investigate exploratory population pharmacokinetics.
5. To investigate changes in clinical status, as measured by change in body weight, Karnofsky Performance Score, disease progression, and development of opportunistic infections.
6. To assess the effect that the different treatment arms have on the patient's quality of life using a standardized instrument. To determine the development of HIV resistance to Ro 31-8959, ddC and ZDV.

Comments:

After this study was initiated, HIV-RNA gained importance as a possible surrogate marker for HIV staging and progression. Therefore,

investigation of changes in this measurement was considered as a primary objective as well.

Although this trial collected endpoints on the development of disease progression and opportunistic infections, this study was designed as a surrogate marker trial. It was not sufficiently powered to detect differences in development of first OI or death.

8.1.1.1.2 Design

The study was a multicenter, double-blind, randomized, phase 2, parallel study sponsored by the AIDS Clinical Trials group (ACTG) of the NIH. Approximately 300 HIV-infected males and females, previously treated with at least 4 months of ZDV and with CD_4 counts between 50 and 300, were randomized to one of the following three arms and treated for 24 weeks:

1) SAQ - 600 mg tid
   ZDV - 200 mg tid
   ddC - 0.75 mg tid

2) SAQ - 600 mg tid
   ZDV - 200 mg tid

3) ZDV - 200 mg tid
   ddC - 0.75 mg tid

Comments:
At the time this protocol was initiated clinical utility of the combination ZDV + ddC (the control arm) relative to ZDV monotherapy had not been confirmed. Later the results of ACTG 155 were unable to show, in the patients studied, the clinical benefit of ZDV+ddC compared to ZDV monotherapy. In ACTG 155, mean duration of prior ZDV use was 18 months. Commenting on the lack of overall difference in clinical outcome between treatment arms in ACTG 155, the investigators hypothesized that those with extensive prior ZDV use may have been less likely to respond. Fischl et al state, "Because long-term zidovudine therapy is associated with resistance and zidovudine resistance is associated with a greater risk for disease progression, adding a single nucleoside agent may be unlikely to produce synergistic anti-HIV

activity."[1] Like ACTG 155, ACTG 229 permitted the enrollment of individuals with prolonged prior use of ZDV. A protocol amendment also allowed the inclusion of patients with prior use of ZDV+ddC and other antiretrovirals such as ddI. This complicates the interpretation of results from this study.

Blinding
After the initial 24 week period, patients were allowed to continue their randomized treatment in a blinded manner in a protocol extension. Blinded study medication was provided until March 31, 1994. After unblinding subjects were allowed to participate in rollover protocol NV14802.

Randomization and Stratification
Each ACTG unit registered patients by computer link to a central computer with access to the "Patient Randomization Program." Subjects were stratified according to prior ZDV + ddC treatment (< 1 week and ≥ 1 week ZDV + ddC use).

8.1.1.3 Population
Subjects were recruited from ten ACTG units listed in Appendix 1.

Inclusion Criteria
Eligible subjects were HIV infected males or females, age 13 years or older, with CD4 counts between 50 and 300 cells/mm^3 who were previously ZDV treated, (without toxicity) for at least 4 months (sequential or cumulative), alone or in combination with other antiretroviral therapy. Subjects were required to have acceptable baseline hematology and chemistry labs as specified in the protocol

Exclusion Criteria [Source CANDA final study report (FSR) NV14255/ACTG229, section 2.43 page 16]

- Previous treatment with a HIV proteinase inhibitor
- any antiretroviral agent (except ZDV) or immunomodulatory therapy within 14 days before study start
- current treatment with any investigational agent, antineoplastic agent or radiotherapy (excluding local skin radiotherapy)
- pregnant or lactating women, or those who wish to become pregnant during the course of the study
- males and females of reproductive potential who are unwilling to use an effective method of contraception
- inability to comply with the protocol
- acute serious opportunistic infections, requiring immediate treatment and including (but not limited to) tuberculosis, CMV, cryptococcal meningitis, disseminated MAC, cerebral

[1] Fischl MA, Stanley K, et al Combination and Monotherapy with Zidovudine and Zalcitabine in Patients with Advanced HIV Disease Ann Intern Med 1995, 122:24-32

toxoplasmosis, and P. carinii pneumonia
- known intolerance to any of the three drugs to be administered in this study
- any symptoms suggestive of pancreatitis
- moderate or severe peripheral neuropathy as evidenced by discomfort from numbness, tingling, burning or pain of the extremities, or any related symptoms accompanied by an objective finding
- visceral Kaposi's sarcoma (KS), and/or lymphoma requiring therapy within 6 months of study start
- transfusion dependency
- malabsorption, severe chronic diarrhea (four or more watery bowel movements per day for two weeks prior to start of study), or inability to take adequate oral intake (inadequate intake was defined as unable to eat one or more meals per day) because of chronic nausea, emesis, or abdominal/oral esophageal discomfort.
- treatment with other investigational compounds (patients who met the USPHS recommendation for MAC prophylaxis were permitted to co-enroll into ACTG 196, or treatment with rifabutin could be initiated at the investigator's discretion)
- treatment with other anti-HIV drugs, biological response modifiers (EPO and G-CSF permitted), systemic cytotoxic chemotherapy, or radiation therapy (localized therapy to skin permitted)
- drugs suspected to cause systemic toxicity (myelosuppressive, hepato-, nephro- or neurotoxic- drugs) and those suspected to cause peripheral neuropathy or pancreatitis
- treatment with foscarnet or ganciclovir.

Concomitant Medication
All concomitant medications were to be kept to a minimum and recorded on the CRF if taken within 30 days prior to study entry or during the study. Methadone maintenance was permitted

8.1.1.4 Protocol procedures
After the first 4 weeks subjects were followed monthly for signs and symptoms, chemistry and hematology laboratory assessments, and measurement of T cell subsets Virologic measurements included DNA and RNA PCR, qualitative plasma viral cultures, and quantitative HIV-1 cell culture.

Pharmacokinetics
Samples were obtained for individual pharmacokinetics at three centers (University of Alabama, Birmingham, AL, Stanford University, Stanford, CA, and University of Washington, Seattle, WA). Samples were to be collected during week 1 and week 12 from approximately 35 patients per center. Samples for population PK assessments were collected on all other subjects at clinic visits during weeks 1, 12, and 24.

8.1.1.5 Endpoints
Primary
The primary efficacy endpoints were changes in absolute CD4 counts (cells/mm^3) and PBMC virus culture titers (cellular viremia).

The PBMC culture data were provided to Roche as infectious units per milliliter (IUPM). According to the ACTG methods, the lowest possible value from the PBMC viral culture was 0.22 IUPM PBMC, the highest was 7835 IUPM PBMC.

Secondary
Secondary endpoints included: changes in HIV-RNA (assessed by Roche RCP method and Chiron bDNA methods), serum β-2 microglobulin, CD4/CD8 ratio, serum neopterin, CD4 percent, absolute CD8 lymphocyte count, p24 antigenemia, AIDS-defining opportunistic infection (OI) or death, weight, Karnofsky Performance Score, quality of life, and assessment of pharmacokinetic parameters for the three drugs.

The protocol defined the efficacy and safety analysis populations as follows:

Safety—at least one dose of trial medication plus one post-baseline safety follow-up.
Intent-to-Treat—at least one dose of trial medication plus one baseline and one post-baseline assessment for either CD4 or PBMC

Safety
Investigators evaluated the intensity of each adverse event as mild, moderate, severe, or potentially life-threatening, corresponding to ACTG protocol grades 1 to 4, respectively

Adverse events were classified by the investigator as due to HIV infection, protocol treatment, neither HIV nor protocol treatment, both HIV and protocol treatment, or unable to judge. To provide consistency with other SAQ trials, Roche reclassified these assessments to probably drug-related, remotely drug-related or unrelated to test drug, see Table 2.

Table 2. Roche classification for assessing adverse event relationships

Investigator Classification	Probable	Remote	Unrelated
HIV Infection			X
Protocol Treatment	X		
Neither HIV nor Protocol Treatment			X
Both HIV and Protocol Treatment	X		
Unable to Judge		X	

Source: CANDA FSR for NV14255/ACTG229, GCR N-130855, pg 24.

8.1.1.6 Statistical considerations

The randomization code was unblinded before RNA PCR results were available. To maintain the study blinding, the randomization codes and results

of the other analyses were not disclosed to the scientists performing these assessments.

Definition of baseline
Baseline was calculated as the mean of the screening (when available), pre-entry and Day 1 (week 0) assessments.

Sample Size
This trial was designed to detect a difference in CD4 change equal to 0.5 times the standard deviation (85% power and $\alpha = 0.05$). The sample size calculation was adjusted for an estimated 15% of subjects being lost to follow-up or nonevaluable.

Protocol -specified analyses of changes in CD4
The protocol states that the absolute CD4 count will be analyzed by calculating the slopes of the log-transformed values for each patient. Comparison of slopes was to be accomplished using an ANCOVA with baseline CD4 as a covariate and treatment and center as the two factors.

Analyses of changes in virologic parameters
The average $TCID_{50}$ of PBMC was compared between treatments using ANCOVA with baseline TCID50 and centers as covariates.
In the analysis, if a post-baseline assessment was at or below the lower limit of detection of the assay, the result was set to the assay lower limit in the calculation of the mean

Comment:
Roche analyzed both CD4 and HIV-RNA PCR measurements comparing mean changes from baseline over time using the DAVG metric, as requested by our review team. This method is described below in the results section

8.1.1.7 Results
The first subject was enrolled on March 1, 1993; the last subject completed 24 weeks of study therapy on December 31, 1993. Extension of double-blind therapy was allowed until the common closing date of March 31, 1994. This provided an additional 12 weeks (at least) of double-blind data. Some patients subsequently received open-label SAQ.

8.1.1.7.1 Patient Disposition
A total of 302 subjects were enrolled in the study. Table 3 shows the number of patients randomized to each study arm and the number evaluable for the safety and intent-to-treat (ITT) analyses. Three patients who were randomized never received study medication.

Table 3. Overview of (FDA) Analysis Populations and Reasons for Exclusion From Analyses Populations.

TREATMENT	ZDV+DDC*	ZDV+SAQ	ZDV+DDC+SAQ
TOTAL NUMBER OF PATIENTS RANDOMIZED	101	101	100
REASONS FOR EXCLUSION FROM ANALYSIS POPULATION			
LOST TO FOLLOW-UP	0	2	0
NOT TREATED	1	0	2
TOTAL NUMBER IN SAFETY POPULATION	100	99	98
REASONS FOR EXCLUSION FROM ANALYSIS POPULATION			
NO PRIMARY EFFICACY DATA POST-BASELINE	1	1	1
TOTAL NUMBER IN INTENT TO TREAT POPULATION	99	98	97

*Does not include patient no 1101/0054 who was randomized to ZDV-ddC but received Ro 31-8959-ZDV-ddC
Source: CANDA FSR for NV14255/ACTG229, GCR-N-130855, pg 35

Eleven patients in the ZDV+ddC group had measurable levels of SAQ in plasma samples. Roche ascertained that a pharmacist mistakenly dispensed saquinavir capsules instead of placebo to one patient (1101/0054) A second patient (2701/0133) received the triple combination in error at week 1 instead of ZDV+ddC but received the correct medication for the rest of the study. Both subjects were analyzed in the ZDV+ddC group in the ITT analyses. Possible reasons for measurable SAQ levels in the nine remaining subjects were: sample mislabelling, contamination, or drug sharing. All patients were included in the ITT population and analyzed according to randomization. Tables 4 and 5 list the number (percentage) of individuals withdrawing over the first 24 weeks of study and entire study, respectively. Reasons for study withdrawal are included A total of 251 subjects completed the first 24 weeks Across treatment arms the number (percentage) completing 24 weeks was 81 (80%) randomized to ZDV+ddC, 83 (82%) to ZDV+SAQ and 87 (87%) to ZDV+ddC+SAQ The table below lists the number (percentage) of individuals withdrawing over the entire duration (including the double-blind extension phase) of the study and reasons for withdrawal.

Table 4. Summary of Early Terminations by Trial Treatment (Safety Population) for first 24 weeks.

REASON FOR PREMATURE WITHDRAWAL	ZDV+ddC N = 100		ZDV+SAQ N = 99		ZDV+ddC+SAQ N = 98	
	No.	(%)	No.	(%)	No.	(%)
TOTAL PATIENTS WITH PREMATURE WITHDRAWAL	19	(19)	17	(16)	11	(11)
ADVERSE EVENT/INTERCURRENT ILLNESS	10	(10)	11	(11)	9	(9)
PATIENT REQUEST	3	(3)	4	(3)	0	(0)
NONCOMPLIANCE	3	(3)	0	(0)	1	(1)
LOST TO FOLLOW-UP	1	(1)	1	(1)	1	(1)
PROTOCOL VIOLATION	0	(0)	1	(1)	0	(0)
PREGNANCY	1	(1)	0	(0)	0	(0)
UNKNOWN	1	(1)	0	(0)	0	(0)

Source: CANDA FSR for NV14255/ACTG229, GCR-N-130855 pg. 36
N = # available in safety population

Table 5. Summary of Early Terminations by Trial Treatment (Safety Population) during entire study.

REASON FOR PREMATURE WITHDRAWAL	ZDV+ddC N = 100		ZDV+SAQ N = 99		ZDV+ddC+SAQ N = 98	
	No	(%)	No.	(%)	No.	(%)
TOTAL PATIENTS WITH PREMATURE WITHDRAWAL	32	(32)	25	(25)	23	(23)
ADVERSE EVENT/INTERCURRENT ILLNESS	14	(14)	15	(15)	13	(13)
PATIENT REQUEST	6	(6)	7	(7)	5	(5)
NONCOMPLIANCE	7	(7)	1	(1)	1	(1)
TREATMENT FAILURE	2	(2)	0	(0)	1	(1)
LOST TO FOLLOW-UP	1	(1)	1	(1)	3	(3)
PROTOCOL VIOLATION	0	(0)	1	(1)	0	(0)
PREGNANCY	1	(1)	0	(0)	0	(0)
UNKNOWN	1	(1)	0	(0)	0	(0)

Source CANDA FSR for NV14255/ACTG229, GCR-N-138384 pg. 30, table 4.

8.1.1.7.2 Patient comparability

Treatment arms were comparable with regard to sex, age and weight of the subjects More african-americans were enrolled in the ZDV+ddC group (16%) compared to 11% and 6% for the ZDV+SAQ and ZDV+ddC+SAQ groups, respectively

Table 6 shows selected baseline characteristics. Patients randomized to the triple combination group had slightly lower baseline CD4 counts (mean, median), however these differences are not clinically relevant. Patients randomized to the triple combination also had slightly higher levels of "viral load" as measured by PBMC and RNA-PCR. The median duration of prior ZDV therapy was longest in the ZDV+SAQ group, however all groups had prolonged prior ZDV treatment (approximately 650 days). Table 7 shows the percentage of patients receiving other antiretrovirals prior to randomization.

Table 6. ACTG 229. Baseline Characteristics of ITT Analysis population

Treatment Group	ZDV+ddC N=100	ZDV+SAQ N=98	ZDV+ddC+SAQ N=97
	N (%)	N (%)	N (%)
Diagnosis			
AIDS	11 (11.0)	10 (10.2)	13 (13.4)
Symptomatic (Non-AIDS)	48 (48.0)	51 (52.0)	51 (52.6)
Asymptomatic	41 (41.0)	37 (37.8)	33 (34.0)
Prior ZDV			
Number Patients	99	98	97
Median Duration (Days)	659	717	647
Prior ZDV-ddC			
Number Patients	33	40	41
Median Duration (Days)	258	195	168
CD4 Count			
Median CD4 Count	169	155	146
Mean CD4 Count	174	163	157
PBMC			
Median $\log_{10}$ PBMC (log IUPM)	1.3	1.4	1.5
RNA PCR (RBL)*			
Median RNA PCR (RBL) (copies/ml)	64840	78290	82980
Median $\log_{10}$ transformed	4.8	4.9	4.9
Plasma Viremia			
Plasma Viremia Positve	38	46	43

*Roche Biomedical labs
Source CANDA FSR for NV14255/ACTG 229, GCR-N-130855, pg. 33., Table 5.

Table 7. Number (percentage) of patients receiving other antiretroviral drugs prior to randomization.

OTHER ANTIRETROVIRALS	ZDV-DDC N = 100		ZDV+SAQ N = 99		ZDV+DDC+SAQ N = 98	
	NO.	(%)	NO.	(%)	NO.	(%)
ZIDOVUDINE	97	(97.0)	98	(99.0)	97	(99.0)
ZALCITABINE	37	(37.0)	46	(46.5)	43	(43.9)
DIDEOXYINOSINE	36	(36.0)	31	(31.3)	32	(32.7)
STAVUDINE	3	(3.0)	2	(2.0)	2	(2.0)
NEVIRAPINE	-		-		1	(1.0)

Source: CANDA FSR for NV14255/ACTG229, GCR-N-130855, pg. 223, Appendix VIII

8.1.1.7.3 Activity endpoint outcomes

The ACTG specified analysis for evaluating absolute CD4 cell changes was a comparison of log-transformed slopes. This metric did not provide a good fit for the data. Roche and FDA analyzed CD4 counts using the DAVG metric (the weighted average of the mean change from baseline over time). This type of analysis has been used to evaluate surrogate responses with other antiretroviral drugs. Although the protocol primary analysis period for this study was 24 weeks, FDA analyzed CD4 and RNA changes over 16 weeks to allow comparability of results with the other four SAQ trials. The 16 week analysis is included in this review and was presented at the advisory committee meeting (Nov 7, 1995). The DAVG-16 and DAVG-24 changes in markers are quite similar and overall conclusions are the same. The label includes both 16 and 24 week values.

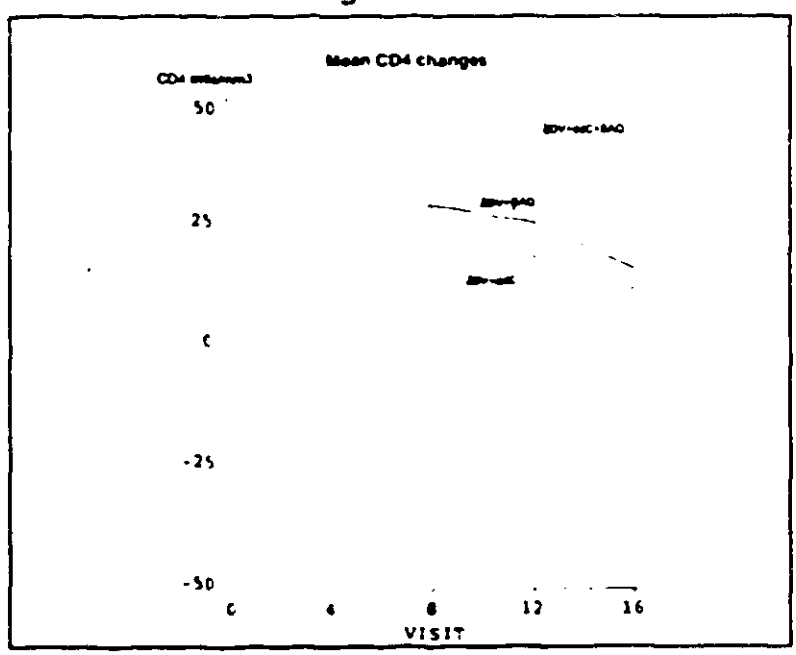
Table 8 shows mean and median CD4 changes from baseline at each visit during the primary analysis period for the ITT population. Figure 1 is a plot of the mean CD4 changes from baseline over 16 weeks.

Table 8. ACTG 229, ITT population. Mean/median CD4 cell change from baseline.

VISIT/WEEK	4	8	12	16	24	32	40	48*
SAQ 600mg-ZDV								
N	96	89	88	89	87	80	75	64
Mean	22	29	26	16	16	-4	-17	-26
Std Dev	52	57	47	42	60	57	56	64
Median	18	16	22	20	10	-9	-14	-25
SAQ 600mg-ZDV-DDC								
N	95	95	87	90	89	83	75	58
Mean	39	43	44	30	26	17	15	14
Std Dev	64	42	72	54	78	62	68	62
Median	32	38	26	25	11	10	1	9
ZDV-DDC								
N	98	95	88	92	87	77	68	58
Mean	18	18	21	13	5	2	-7	-5
Std Dev	61	60	60	73	65	81	76	82
Median	8	7	15	-2	-6	-7	-11	-21

*Italics represents double-blind extension phase
Source: generated from statistical applications of the CANDA
Baseline is median of screening visits and day 1

Figure 1. Plot of Mean CD4 changes from baseline over 16 weeks



Source Kazem Kazempour, SAS applications

For simplicity, only FDA analyses of changes in surrogate markers will be

presented (except where otherwise indicated) in this review. Roche's analyses were similar in all cases and overall conclusions were the same. Table 9 shows the mean CD4 change from baseline averaged over 16 weeks (DAVG-16) for each study treatment. As shown in Fig. 1, the triple combination produced greater mean change in CD4 than the two double combinations.

Table 9. ACTG 229. FDA ITT analysis of mean CD4 changes from baseline over averaged over 16 weeks (DAVG-16).

TREATMENT	N	Mean	95% C.I.
SAQ+ZDV+ddC	97	32	20-44
SAQ+ZDV	98	19	7-31
ZDV+ddC	99	12	0-24

Source: Kazem Kazempour SAS applications

The three treatments were statistically different with respect to CD4 change from baseline. There does not appear to be any significant differences between centers with respect to CD4 outcome (see Table 10).

Table 10. ACTG 229, FDA ITT analysis of CD4 change from baseline (DAVG-16). Overall comparisons

Source	DF	Type III SS	F Value	Pr > F
Treatment	2	19297.15	5.40	0.0050
Center	15	15056.82	0.56	0.9028

Source Kazem Kazempour SAS applications

Pairwise comparisons are shown in Table 11. Comparisons were adjusted using Scheffe's test to control the type I experiment-wise error rate for all pairwise comparisons. This method will be used in all subsequent analysis included in this review.

The only pairwise comparison of CD4 change achieving statistical significance is the superiority of the triple combination compared to ZDV+ddC. Therefore, a SAQ containing regimen produced greater increases in CD4 counts than the regimen without SAQ. At present it is unknown whether these increases are clinically relevant.

Table 11. ACTG 229 ITT population. FDA pairwise comparisons for CD4 DAVG-16 analysis.

TREATMENT	Difference in Means	95% C.I.
Triple vs ZDV+SAQ	13	-2, 28
Triple vs ZDV+ddC	20	5, 35
ZDV+SAQ vs. ZDV+ddC	7	-7, 22

Source: Kazem Kazempour SAS applications

HIV-RNA

Table 12 shows the mean/median changes from baseline in log transformed HIV-RNA over 24 weeks. The triple combination produced the greatest and most sustained reductions in RNA over this time period. Patients receiving ZDV+SAQ had the least RNA reductions with mean changes returning to baseline by 24 weeks. Figure 2 is a plot of mean HIV-RNA changes over 24 weeks.

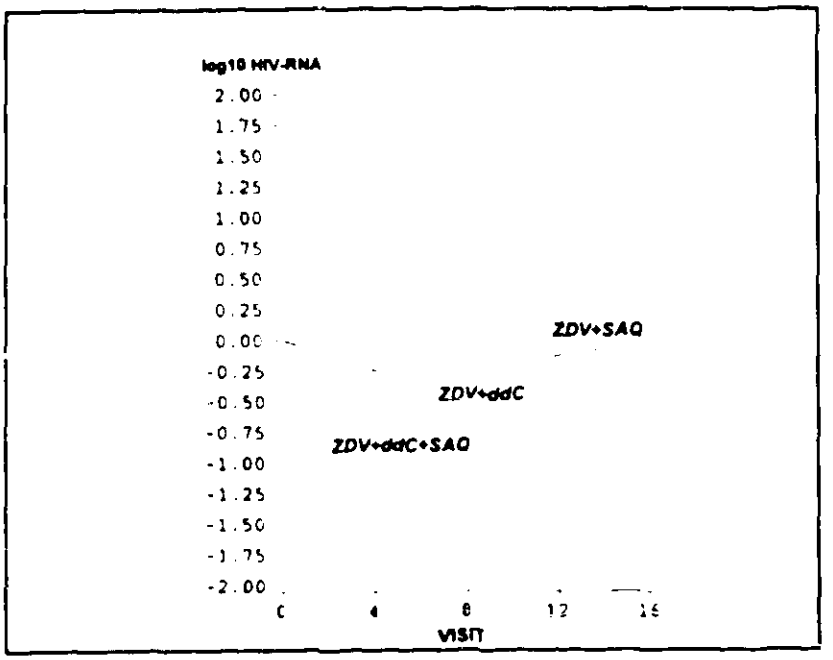
Table 12. ACTG 229 ITT population. Mean/median changes in log transformed HIV-RNA.

VISIT/WEEK	4	8	12	16	24
SAQ 600mg+ZDV					
N	91	85	88	81	83
Mean	-0.2	-0.2	-0.1	0.0	0.0
Std Dev	0.6	0.6	0.6	0.6	0.6
Median	-0.1	-0.2	-0.1	0.0	0.0
SAQ 600mg+ZDV+DDC					
N	92	91	85	86	84
Mean	-0.7	-0.7	-0.6	-0.5	-0.5
Std Dev	0.7	0.7	0.7	0.7	0.8
Median	-0.6	-0.7	-0.7	-0.5	-0.5
ZDV+DDC					
N	98	90	89	93	86
Mean	-0.3	-0.3	-0.3	-0.3	-0.2
Std Dev	0.6	0.7	0.6	0.6	0.7
Median	-0.3	-0.2	-0.1	-0.2	-0.2

Source CANDA

Baseline is Median of Screening Visits and Day 1

Figure 2. ACTG 229. Mean Log_{10}RNA changes from baseline.



Source Kazem Kazempour, SAS applications

Table 13 shows the mean change in HIV-RNA (log transformed copies/mL) by treatment group

Table 13. ACTG 229. FDA ITT analysis of changes in HIV-RNA (DAVG-16).

TREATMENT	N	Mean	95% C.I.
SAQ+ZDV+ddC	95	-0.6	-0.7, -0.4
SAQ+ZDV	94	-0.2	-0.3, 0
ZDV+ddC	99	-0.3	-0.4, -0.1

Source Kazem Kazempour, SAS applications
NOTE Due to missing values, only 288 subjects are included in this analysis.

Overall, there were statistically significant differences in the three treatment arms with respect to change in log transformed HIV-RNA. There are no significant differences between centers with respect to changes in RNA (See Table 14)

Table 14. ACTG 229. FDA ITT analysis. Overall comparisons.

Source	DF	Type III SS	F Value	Pr > F
Treatment	2	8.67	20.35	0.0001
Center	15	4.14	1.30	0.2041

Table 15 shows pairwise comparisons for change in HIV-RNA among treatment groups The triple combination produced statistically significant decreases in HIV-RNA compared to either double combination therapy. There were no statistically significant differences between the two double combinations, ZDV+ddC and ZDV+SAQ.

Table 15. FDA Pairwise comparisons for CD4 DAVG-16 analysis--ITT population ACTG 229

TREATMENT	Difference in Means	95% C.I.
Triple vs ZDV+SAQ	-0 4	-0.6, -0.2
Triple vs ZDV+ddC	-0 3	-0.5, -0.1
ZDV+SAQ vs. ZDV+ddC	-0 1	-0.3, 0.1

Source Kazem Kazempour, SAS applications

Viral PBMC Co-culture

The primary virologic marker specified in the protocol was PBMC viral co-culture As this trial was conducted, plasma RNA as measured by PCR and bDNA gained favor among the scientific community as a surrogate marker in HIV trials

Table 16 shows mean and median changes from baseline in cell-associated viremia over a 24 week period These changes are consistent with that observed for HIV-RNA Appendix III shows the Roche DAVG-16_week analysis for change in cell associated viremia

Table 16. ACTG 229: Summary of log(Cell-associated viremia) - Change from Baseline, ITT Population.

VISIT\WEEK	4	8	12	16	24
SAQ 600mg-ZDV					
N	93	87	91	90	84
Mean	0	0	0	0.1	0.1
Std Dev	1.0	1.0	0.9	0.9	1.0
Median	-0.1	0.0	-0.1	0.0	0.1
SAQ 600mg-ZDV-DDC					
N	92	95	87	90	84
Mean	-0.8	-0 7	-0.6	-0.6	-0.5
Std Dev	0.9	0.9	1.0	1.0	1.2
Median	-0.6	-0 7	-0.7	-0.6	-0.4
ZDV-DDC					
N	98	94	90	95	85
Mean	-0.3	-0 4	-0.3	-0.2	-0.2
Std Dev	0.9	1 1	0.9	0.9	1.2
Median	-0.2	-0 3	-0.3	0.0	0.0

Source: CANDA. Baseline is Median of Screening Visits and Day 1.

Clinical Endpoints

Table 17 shows the endpoints (and date these endpoints occurred relative to initiation of treatment) through the end of the double blind extension phase. There were seven clinical endpoints in the ZDV+ddC group, eight in the ZDV+SAQ group and three endpoints in the triple combination group. There were insufficient number of endpoints to conduct statistical analyses.

Table 17. ACTG 229. Listing of Clinical End-Points

Treatment Group	Center Number	Patient Number	End-Point	Relative Start Day
SAQ-ZDV	401	42300	Lymphoma	63
	1101	110451	Candidiasis, Other	85
	1401	140841	PC Pneumonia, Clinical Diagnosis	63
	2301	230139	DEATH*	50
	2705	271020	Candidiasis, Oral, Presumptive	113
	5801	580042	Small Non-Cleaved Lymphoma	100
	6301	630080	DEATH **	124
	401	2154	K.S	275
SAQ-ZDV-DDC	401	40835	PC Pneumonia, Other	44
	502	50678	PC Pneumonia, Clinical Diagnosis	179
	1101	0876	CMV Retinitis	202
ZDV-DDC	401	42310	Candidiasis, Other	88
	501	50692	Cryptosporidiosis, Other	88
	1401	140135	PC Pneumonia, Clinical Diagnosis	140
	6201	620161	CMV, Esoph	28
	502	0717	PCP-Lung	274
	1401	0568	DEATH	269
	1401	0667	Esophageal candidiasis	198

Source: CANDA FSR for NV14255/ACTG229, GCR-N-130'384, Vol. 1 pg. 61, Table 28.
*lymphoma at week 4, patient died in week 8
**lymphoma at week 1, patient died in week 17

8.1.1.7.4 Safety comparisons

Extent of Exposure

Table 18 shows the number of individuals continuing study drug in each treatment arm for given time periods. Greater than 85% of the subjects randomized received 20 to 24 weeks of study drug. Median duration of study treatment was approximately 48 weeks. There appeared to be an increase in treatment discontinuations for the ZDV+ddC group compared to the other groups, especially after 40 weeks.

Table 18. Summary of exposure to study treatment. ACTG 229: Safety Population

Treatment	ZDV-DDC* N=100	SAQ-ZDV N=99	SAQ-ZDV-DDC N=98
Treatment Duration (weeks)			
0 - 4	100	99	98
>4 - 8	96	94	95
>8 - 12	91	94	93
>12 - 16	90	92	90
>16 - 20	88	87	89
>20 - 24	86	85	89
>24 - 28	80	80	84
>28 - 32	74	79	81
>32 - 36	73	76	78
>36 - 40	72	76	78
>40 - 44	68	76	77
>44 - 48	60	70	72
>48 - 52	39	48	53
>52 - 56	18	19	23
>56 - 60	0	2	1
>60 - 64	0	0	0

*One patient (1101/0054) in this group actually received Ro 31-8959-ZDV-ddC in error

Source: CANDA FSR for NV14255/ACTG 229, GCR N-130'855, page 84, table 48.

Adverse Events

Tables 19 and 20 list the percentage of individuals experiencing adverse events of moderate or greater intensity among the three treatment arms. All adverse events were considered to have a probable relationship; for study ACTG 229, there was a no "possible relationship category" (please refer to Table 2 for assignment of relationship). Table 19 includes adverse events of all intensities (with a probable relationship). Fewer patients receiving ZDV+SAQ experienced at least one adverse event compared to those receiving either ZDV+ddC or the triple combination. The most frequently reported adverse events among patients receiving SAQ involved the *gastrointestinal body system*. *diarrhea*. *abdominal discomfort* and *nausea* were the most frequent specific events. A greater number of patients on ddC containing regimens experienced paraesthesia and numbness in the extremities compared to the ZDV+SAQ arm.

Table 19. ACTG 229. Number (percentage) of Patients with Adverse Events
Intensity: Moderate or Severe in Intensity; Relationship: probably related
Listed by type if frequency ≥3% in any treatment arm

BODY SYSTEM /ADVERSE EVENT	ZDV+ddC N = 100		ZDV+SAQ N = 99		ZDV+ddC+SAQ N = 98	
	No.	(%)	No.	(%)	No.	(%)
ALL BODY SYSTEMS						
Total patients with at least one AE	27	(27.0)	11	(11.1)	23	(23.5)
Total number of AEs	62		32		44	
GASTRO-INTESTINAL SYSTEM DISORDERS						
ABDOMINAL DISCOMFORT	4	(4.0)	2	(2.0)	3	(3.1)
NAUSEA	3	(3.0)	-		3	(3.1)
DIARRHEA	-		3	(3.0)	1	(1.0)
STOMATITIS	4	(4.0)	-		-	
BODY AS A WHOLE - GENERAL DISORDERS						
ASTHENIA	10	(10.0)	6	(6.1)	9	(9.2)
CENTRAL & PERIPHERAL NERVOUS SYSTEM						
PARESTHESIA	4	(4.0)	2	(2.0)	3	(3.1)
NUMBNESS EXTREMITIES	4	(4.0)	2	(2.0)	1	(1.0)
MUSCULO-SKELETAL SYSTEM						
PAIN MUSCULO-SKELETAL	4	(4.0)	2	(2.0)	2	(2.0)
MYALGIA	3	(3.0)	1	(1.0)	-	
SKIN AND APPENDAGES						
RASH	3	(3.0)	-		-	

Source CANDA FSR for NV14255/ACTG 229, GCR N-138'384, pg 76, table 32.

Table 20. ACTG 229. Percentage of Patients with Adverse events
Relationship: probable; Intensity: ALL= Mild, Moderate or Severe.
Listed by type if frequency ≥3% in any treatment arm

BODY SYSTEM /ADVERSE EVENT	ZDV-ddC N = 100	SAQ-ZDV N = 99	SAQ-ZDV-ddC N = 98
ALL BODY SYSTEMS			
Total patients with at least one AE	51	28	43
Total number of AEs	126	84	90
GASTRO-INTESTINAL SYSTEM DISORDERS			
ABDOMINAL DISCOMFORT	13	10	16
NAUSEA	11	4	6
DIARRHEA	2	7	4
STOMATITIS	8	1	8
DYSPEPSIA	4	4	2
ABDOMINAL PAIN	2	3	2
BODY AS A WHOLE - GENERAL DISORDERS			
ASTHENIA	12	7	9
APPETITE DISTURBANCE	4	1	1
CENTRAL & PERIPHERAL NERVOUS SYSTEM			
PARESTHESIA	8	6	4
NUMBNESS EXTREMITIES	6	2	5
HEADACHE	7	8	4
DIZZINESS	3	-	4
MUSCULO-SKELETAL SYSTEM			
PAIN MUSCULO-SKELETAL	6	5	3
MYALGIA	4	1	-
SKIN AND APPENDAGES			
RASH	5	-	-
PSYCHIATRIC DISORDERS			
EUPHORIA	2	4	1
INSOMNIA	1	3	1

Source: generated from SAS application of the CANDA

In Tables 19 and 20, the term stomatitis replaces Roche's terms, "buccal mucosal ulceration" and "mucosal damage." The percentage of individuals experiencing stomatitis was determined by combining these terms and recalculating using the SAS application of the CANDA.

Deaths and Serious Adverse Events

Table 21 lists the number (percentage) of patients dying or experiencing a

serious adverse event during the entire study period. Events are reported according to their relationship to study treatment (as assessed by the investigators).

Table 21. ACTG 229. Serious Adverse Events During the entire study period.
Double blind and open label phase

	ZDV+ddC N=100	ZDV+SAQ N=99	ZDV+ddC+SAQ N=98
Deaths - Related	0	0	0
Deaths - Unrelated	2 (2%)	3 (3%)	0
Serious - Possibly or Probably Related	4 (4%)	3 (3%)	1 (1%)
Serious - Remotely Related	0	1 (1%)	0
Serious - Unrelated	11 (11%)	10 (10%)	11 (11%)
Overdose	1 (1%)	0	1 (1%)

Source: CANDA FSR for NV14255/ACTG 229, GCR N-130'384 pg.80, Table 3.

All five deaths (3 on the ZDV+SAQ arm and 2 on the ZDV+ddC arm) were considered to be unrelated to study drug The cause of death for three patients was listed as lymphoma

Five serious adverse events were at least remotely related to ZDV+SAQ or ZDV+ddC+SAQ. The four serious adverse events that were considered to be at least remotely related to ZDV+SAQ combination were:

1) Pt #1401/0219 was hospitalized for a constellation of symptoms including, severe weakness, fever, confusion, incontinence, ataxia, orthostatic hypotension and increased epigastric tenderness.

2) Pt # 0401/2330 developed fatigue, nausea, and elevation of liver function tests

3) Pt # 0401/2303 developed acute myeloblastic leukemia. He had been off study drug for over 2 months

4) Pt # 1101/0786 developed elevated transaminases; this did not recur on rechallenge

The serious event that was remotely related to triple therapy was an elevation of transaminases. This patient had grade 2 transaminase elevation at baseline and developed grade 3 transaminase elevation at week 48.

In addition to the above serious adverse events considered at least remotely

related to drug, a patient on ZDV+SAQ developed pancreatitis that was considered unrelated to study treatment but possibly related to alcohol. Except for this patient, no other patient had evidence of clinical pancreatitis or characteristic abdominal pain in association with elevated amylase.

Adverse events leading to premature discontinuation of study treatment

Through the double blind/open label phase, a total of 42 patients discontinued study treatment for an adverse event or intercurrent illness. Table 22 lists the number (percentage) of patients in each treatment group with adverse events leading to premature discontinuation of study treatment. Adverse events in the SAQ containing arms that were considered possibly or probably related to study treatment included nausea, abdominal discomfort, metallic taste, vomiting, belching and flatulence, constipation, hiccoughs, fatigue, neutropenia, peripheral neuropathy, headaches, dizziness and light headedness, disruptive mood swings, agitation, decreased concentration, urinary hesitancy, and apthous stomatitis. Some of these events (specifically, neutropenia, fatigue, neuropathy, headaches, and stomatitis) are known toxicities of ZDV and/or ddC.

Table 22. ACTG 229. Adverse Events Leading to Permanent Discontinuation of All Study Drugs During the Entire Study Period.

	ZDV-ddC N=100	SAQ-ZDV N=99	SAQ-ZDV-ddC N=98
Relationship to Treatment			
- Probably or Possibly	12 (12%)	7 (7%)	9 (9%)
- Remotely	0	0	0
- Unrelated	2 (2%)	8 (8%)	5 (5%)
Total	14 (14%)	15 (15%)	13 (13%)

Source CANDA FSR for NV14255/ACTG 229. GCR N-130384, pg. 86, Table 34.

Laboratory Abnormalities

Table 23 lists marked laboratory abnormalities for each of the treatment groups over the duration of the entire study period. Marked laboratory abnormalities were defined as a grade 3 or 4 abnormality in a patient with a normal baseline or a grade 4 abnormality in a patient with a grade 1 baseline.

Table 23. ACTG 229. Summary of Marked Laboratory Abnormalities during the entire study period.

Laboratory Test	Number of Patients With Marked Laboratory Abnormalities		
	ZDV+ddC	SAQ+ZDV	SAQ+ZDV+ddC
Neutrophils (neutropenia)	8	4	2
Hemoglobin (anemia)	1	0	0
SGPT	1	0	3
SGOT	0	2	2
Bilirubin	0	1	0
Serum amylase	1	2	1
Phosphate	0	2	1
Calcium (hypercalcemia)	0	1	0
Low Platelets	2	0	0
High Uric Acid	1	0	0
Total	14	12	10

Source : CANDA Integrated Safety Summary, W-144'973, table 21, pgs 147-155.

8.1.1.8 Reviewer's Conclusions

Subjects participating in this study not only had prolonged prior therapy with ZDV but also with other nucleosides (as monotherapy and in various combinations) More than a third had prior treatment with ZDV+ddC, which is one of the treatment arms This heterogeneity in prior antiviral therapy may have influenced the activity results On should take caution in extrapolating these results to the treatment naive population. Preliminary results from ACTG 175 and the Delta trials indicate that nucleoside combinations may be better than ZDV monotherapy in treatment naive-individuals. However results from these same trials and from ACTG 155 showed that combination therapy does not appear to be better than ZDV monotherapy in individuals who have already had prior ZDV exposure Continued efficacy of ZDV monotherapy after prolonged exposure is also uncertain

Activity

To assess the activity of SAQ as part of a combination regimen, the comparisons of interest in this protocol are the triple combination compared to ZDV+ddC, and ZDV+SAQ compared to ZDV+ddC. Analyses of average change in CD4 and HIV-RNA from baseline demonstrate superior activity of the triple combination compared to ZDV+ddC. This indicates that the addition of SAQ to ZDV+ddC produces greater activity than the combination without SAQ

There were no statistically significant differences between ZDV+SAQ and ZDV+ddC in this study with respect to changes in CD4 or RNA from baseline. Subjects in the ZDV+SAQ had somewhat higher CD4 increases but also slightly less RNA reductions The clinical significance of this discordance of rank order in activity between the two markers in unknown. However, this

type of response was shown in both the North American trial (NV14256) and the Italian study (see below). Possible mechanisms for this are discussed in the conclusions of the N. American trial.

Safety

SAQ does not appear to alter the pattern, frequency, or severity of toxicities known to be associated with ZDV and/or ddC. Most of the adverse events observed in this trial were of mild intensity. Adverse events involving the gastrointestinal system were observed in a higher percentage of patients compared to other body systems. It is difficult to ascribe any particular toxicity or laboratory abnormality to SAQ. There was a slight increase in the frequency of diarrhea. There were also several serious adverse events involving an increase in transaminases; however there did not appear to be a preponderance of marked transaminase elevations on SAQ containing arms compared to the ZDV+ddC control.

8.1.2 NV14256: Surrogate Analysis Cohort

"A randomized, double-blind, multi-center, parallel study of saquinavir (Ro 31-8959) alone, HIVID® (zalcitabine, ddC) alone, and both in combination as treatment for advanced HIV infection (CD4 50-300 cells/mm^3) in patients discontinuing or unable to take Retrovir® (zidovudine, ZDV) therapy."

The study report of NV14256 includes laboratory marker (CD4 lymphocyte and HIV-1 by RNA) and safety data from a surrogate analysis, planned in the protocol, of 451 evaluable patients who had received a minimum of 16 weeks of treatment, or who had dropped out before 16 weeks, to a clinical cut-off date of 2nd January 1995.

8.1.2.1 Protocol

8.1.2.1.1 Objectives

Primary Objectives

The primary objectives of this study for the surrogate analysis cohort were to compare safety, tolerability and activity, based on change from baseline of laboratory markers (change in CD4 cell count, change in plasma viremia as measured by RNA-PCR)

The primary objective for the entire study population is to were to compare the safety, tolerability and efficacy, based on clinical endpoints (first AIDS-defining clinical events or death) of the three treatments.

Secondary Objectives

- To assess the surrogate value of the laboratory markers of activity for clinical endpoints at final analysis
- To describe the development of decreased viral susceptibility to either saquinavir or ddC in each of the study arms
- To describe the incidence of emergence of syncytium-inducing variants in each of the study arms
- To describe changes in clinical status based on serial measurement of weight and Karnofsky score
- To obtain information on the effect of covariates on the pharmacokinetics of saquinavir

8.1.2.1.2 Design

The amended design of this study is a randomized, double-blind, multicenter parallel three-arm phase 2/3 fixed dose study, in HIV-infected patients (CD4 = 50-300 cells/mm3) discontinuing or unable to take ZDV.

The three treatment arms are:

1) ddC 0.75 mg q8h
2) SAQ 600 mg q8h
3) SAQ 600 mg q8h + ddC 0.75 mg q8h

The original protocol design contained a fourth arm, SAQ 200 mg q8h + ddC. However after the study started, new data from the analysis of HIV-1 RNA in the Italian dose-ranging study, V-13330, showed that reduction in viral load in the SAQ 200 mg + ZDV arm was not different to that achieved by ZDV alone. In August, 1994, after discussion with the Data Safety Monitoring Board, Roche discontinued the SAQ 200 mg + ddC arm. One hundred eight patients had been accrued into this arm; these subjects were offered treatment under a separate rollover protocol (SV14788).

Stratification

Randomization was stratified into two groups based on pre-entry CD4 count (<100 cells/mm^3 and ≥ 100 cells/mm^3). No more than 25% of the subjects were to be randomized to the lower stratum.

8.1.2.1.3 Population

HIV-infected patients were enrolled at 49 investigational centers in the U.S. and Canada. Each center was to accrue no less than 12 and no more than 36 subjects. Subjects from 42 centers are included in the surrogate analysis.

Inclusion Criteria

Participants were required to be HIV-infected adults (at least 18) with pre-entry CD4 counts between 50 and 300 cells/mm^3 and a Karnofsky score ≥ 60. Subjects must have had at least 16 weeks of prior ZDV therapy, unless he/she experienced a toxicity requiring discontinuation of ZDV. In addition baseline chemistry and hematology had to be within an acceptable range as specified in the protocol.

Comment:

Onginally the protocol did not require a minimum amount of prior ZDV expenence. However, it was brought to Roche's attention that some individuals may have taken only one day of ZDV before deciding to switch to new therapy. The intent of the inclusion criteria was to enroll those who had discontinued ZDV secondary to toxicity or after ZDV utility was no longer apparent to the patient or physician. Therefore, the protocol was amended to require a minimum of 16 weeks prior ZDV treatment (in the absence of dose-limiting toxicity).

Exclusion Criteria [from NV14256 CANDA Study Report; W-144'954, page 16]

- any history of a grade 2 or worse peripheral neuropathy
- signs and symptoms of peripheral neuropathy as defined by either the Signs and Symptoms Questionnaire (on the peripheral neuropathy segment, a score of moderate in any one category, or a score of mild in two categories) or by neurological examination (any moderate abnormality indicative of peripheral neuropathy, particularly impaired sensation of sharp pain, light touch or vibration in lower extremities, distal extremity weakness, or distal extremity hyporeflexia).
- malabsorption, or an inability to take adequate oral intake (i.e. inability to eat one or more meals a day due to chronic nausea, emesis, or abdominal/oral oesophageal discomfort).
- any grade 3 or greater laboratory or clinical abnormality.
- active opportunistic infection (OI) requiring immediate treatment. Patients with cytomegalovirus retinitis were excluded from the study. Acute therapy was to have been completed at least 14 days before study entry.
- active OI at screening, requiring treatment with a prohibited concomitant medication. If the patient was successfully treated, or remained on chronic suppressive therapy and had remained stable for 2 weeks after treatment, the patient was allowed to enter the study.
- unexplained elevated temperature > 38.5C (101.5F) persisting for 14 days or more within a 28 day period before study entry
- unexplained chronic diarrhoea (> 3 loose stools per day) persisting for 14 days or more within a 28 day period before study entry
- malignancy, visceral Kaposi's sarcoma or lymphoma requiring systemic chemotherapy and/or radiotherapy within the next 48 weeks
- history of non-Hodgkin's lymphoma
- inability to comply with the protocol requirements, for reasons other than those specified.
- pregnant women, breast-feeding women, or women who believed they might wish to become pregnant during the course of the study
- females of reproductive potential who were unwilling to use an effective method of contraception during the study
- unwillingness or inability to sign an informed consent form.
- previous treatment with an HIV protease inhibitor
- prior treatment with ddC, ddI, D4T for greater than two weeks; prior treatment with non-nucleoside reverse transcriptase inhibitors for greater than 8 weeks.
- concomitant or maintenance treatment with excluded medications (e.g. experimental drugs, drugs with known nephrotoxic or hepatotoxic potential, and drugs likely to cause peripheral neuropathy)
- antineoplastic agents or radiation therapy excluding local skin radiation therapy.
- immunomodulators (systemic interferon, candidate vaccines, or other experimental therapies) within 3 months of starting study medication

Use of Mycobacteria avium intracellulare prophylaxis (rifabutin, clarithromycin, azithromycin) and use of rifampicin and ketoconazole was discouraged, due to drug interactions. Patients requiring treatment of cytomegalovirus infections with foscarnet or ganciclovir were withdrawn from the study.

8.1.2.1.4 Study procedures

The eligibility assessments (history, physical exam, laboratory safety tests and CD4 cell count) were performed at least 28 days prior to the pre-entry visit for subjects currently on antiretroviral therapy. For subjects who had not received antiretroviral therapy for at least 28 days, eligibility assessments were made as part of the pre-entry examination. Pre-entry examination included the eligibility

assessments plus a plasma HIV RNA measurement.[2] These assessments/measurements were repeated on day 1 of treatment. Subjects returned for clinic visits and laboratory assessments (chemistry, hematology, CD4 and plasma RNA-PCR) every 4 weeks up to week 16 and then every 8 weeks thereafter. Blood samples for testing drug sensitivity and SI variants, and PBMCs for genotyping were drawn at day 1 and weeks 24 and 48. assessments.

8.1.2.3.3 Endpoints

The primary study endpoint is progression to an AIDS defining event (clinical events according to the 1993 CDC criteria) or death. However, the study protocol also planned to analyze activity parameters (surrogate markers) prior to but without compromising the completion of the evaluation of clinical endpoints.[3] Primary parameters for the surrogate endpoint are absolute CD4 count and quantitative plasma RNA-PCR

8.1.2.3.4 Statistical considerations

The ITT population included all randomized patients with at least one pretreatment assessment and at least one post baseline efficacy assessment. Baseline was defined as the mean of the pre-entry and day 1 assessments for both primary efficacy parameters

A sample size of 450 patients 150 per treatment group was planned for this surrogate analysis Roche's sample size calculations assumed a type 1 error of 0.02 to give an overall study significance level from the three pairwise comparisons of approximately 0.05

Patients were evaluable for safety if they had received at least one dose of trial medication and had at least one follow-up safety assessment. The time window for collection of safety data was up to 28 days following the final dose of either trial treatment The denominator for the calculation of adverse event rates included only those patients for whom post-baseline safety information was available In addition to data obtained from the evaluable patients included in the surrogate cohort serious adverse events, drop-outs due to adverse events and deaths occurring in all patients randomized (surrogate and nonsurrogate cohort up to a common closing date) were also included in the NDA.

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[2]The lower limit of the RNA PCR assay was 200 copies/ml.

[3]Investigators and patients remain blinded to treatment assignment with respect to clinical outcome Only Roche statisticians and FDA reviewers analyzed unblinded data for the surrogate analyses of the surrogate cohort. Clinical endpoints were not evaluated with respect to treatment group.

Marked laboratory abnormalities were defined as a shift in 3 severity grades from baseline; a shift to grade 3 or 4 in a subject with a normal baseline or a shift to grade 4 in a subject with a grade 1 baseline.

Comment:
This is slightly different then the definition used in the three European studies. Marked laboratory abnormalities in these studies were defined as shifts in laboratory values from grade 0 at baseline to a grade 3 or 4 during treatment

Centers
Small centers, those with less than 6 patients, were combined to form geographical regions. Before the randomization code release, 42 centers were grouped into 14 geographical regions.

8.1.2.2 Results

This study report summarized data from 456 randomized patients. All patients who were randomized on or before Sept. 1, 1994, or who had a validated AIDS defining event on or before January 2 1995, were included in this analysis. The minimum treatment period excluding patients who terminated this study prematurely was 16 weeks

8.1.2.2.1 Patient Disposition

Table 24 shows the number of patients who were enrolled into the protocol-specified surrogate marker analysis cohort and the number evaluable for an ITT analysis. Patients with no post-baseline surrogate marker data were excluded.

Comment:
Prior to closing the surrogate analysis cohort data base, Roche decided to exclude the 16 patients from center 14158. This center's primary investigator was being investigated for financial improprieties. The FDA analysis includes patients from this center, since there is no reason to suspect that the surrogate marker data from this center is unreliable.

Table 24 shows that fewer patients were evaluable for HIV-RNA changes than for CD4 changes. Reasons for this discrepancy are discussed in section 8.1.2.2., Efficacy endpoint outcomes, under "additional analyses."

Table 24. NV14256. Overview of FDA Analysis Populations.

TOTAL NUMBER OF PATIENTS IN SURROGATE ANALYSIS	**456**
REASONS FOR EXCLUSION FROM ANALYSIS POPULATION	
NO POST BASELINE EFFICACY AND SAFETY DATA	1
PATIENT RANDOMIZED BUT RECEIVED NO TREATMENT	4
TOTAL NUMBER IN SAFETY POPULATION	**451**
REASONS FOR EXCLUSION FROM ANALYSIS POPULATION	
NO POST BASELINE PRIMARY CD4 DATA	12
TOTAL NUMBER IN INTENT TO TREAT POPULATION FOR CD4	**439**
REASONS FOR EXCLUSION FROM ANALYSIS POPULATION	
NO BASELINE OR POST-BASELINE HIV-RNA DATA	72
TOTAL NUMBER IN INTENT TO TREAT POPULATION FOR RNA	**367**

Source: CANDA study report (SR) for NV14256, W144'954 pg. 30.

Table 25 lists the number (percentage) of subjects prematurely discontinuing study treatment and lists reasons for premature discontinuation. Overall a slightly higher percentage of individuals on the ddC monotherapy arm discontinued treatment prematurely due to a protocol-specified treatment toxicity. However a smaller percentage of individuals on this arm discontinued drug due to reasons listed under the category "patient request."

Table 25. NV14256: Summary of Early Discontinuation of Study Treatment.

REASON FOR PREMATURE WITHDRAWAL	DDC N = 145		SAQ 600 mg N = 159		SAQ 600 mg + DDC N = 147	
	No	(%)	No	(%)	No.	(%)
Total of patents with prem withdrawal	43	(29 7)	38	(23.9)	39	(26.5)
PROTOCOL TREATMENT TOXICITY	12	(8 3)	3	(1.9)	10	(6.8)
NON-PROTOCOL TREATMENT TOXICITY	7	(4 8)	9	(5.7)	4	(2.7)
PATIENT REQUEST	1	(0 7)	8	(5.0)	11	(7.5)
AIDS DEFINING CLINICAL ENDPOINT	7	(4 8)	7	(4.4)	5	(3.4)
LOST TO FOLLOW-UP	6	(4 1)	4	(2.5)	2	(1.4)
OTHER ANTI-RETROVIRAL THERAPY*	5	(3 4)	3	(1.9)	2	(1.4)
MISCELLANEOUS	4	(2 8)	2	(1.3)	3	(2.0)
INVESTIGATOR REQUEST	1	(0 7)	2	(1.3)	2	(1.4)

Source CANDA SR for NV14256 W144'954 page 31

Patent requesting other antiretroviral therapy

8.1.2.4.2 Patient Comparability

Table 26 summarizes demographic data among the three treatment arms. The three treatment groups were comparable with respect to sex, age, weight and race. Mean/median duration of prior ZDV treatment was longer for patients randomized to ddC; however all treatment arms had substantial amounts of prior ZDV. In addition to ZDV therapy, 4 patients in the surrogate analysis population had received prior treatment with ddI, 2 with ddC and 4 with both ddI and ddC. Prior use of other nucleosides constitutes a protocol violation, however these patients were not excluded from the ITT analysis.

Table 26. NV14256: Demographic Data Summary - ITT Population.

TREATMENT	DDC N = 140	SAQ N = 158	SAQ + DDC N = 141
SEX			
n	140	158	141
Males	129 (92%)	149 (94%)	132 (94%)
Females	11 (8%)	9 (6%)	9 (6%)
AGE (y)			
n	139	158	140
Mean	39.0	38.8	38.5
Median	37.0	38.0	38.0
Range	23 - 74	22 - 69	23 - 69
WEIGHT (kg)			
n	136	157	138
Mean	75.3	75.5	74.9
Median	72.3	74.5	73.8
Range	36 - 111	47 - 126	47 - 109
RACE			
n	140	158	141
White	101 (72%)	117 (74%)	108 (77%)
Black	14 (10%)	20 (13%)	15 (11%)
Oriental	3 (2%)	1 (<1%)	1 (<1%)
Hispanic	17 (12%)	20 (13%)	16 (11%)
Other	5 (4%)	0 (0%)	1 (<1%)
TOTAL DURATION OF PRIOR ZDV (days)			
N	145	158	145
Mean	712.9	620.2	651.4
Median	556.0	403.5	435.0

Source: CANDA SR for NV14256, W144954 pg 33, Table 5.

Table 27 shows mean/median baseline CD4 counts and HIV-RNA (as measured by PCR) for each treatment group. As stated above, there were less patients with available RNA measurements

Table 27. **NV14256: Roche ITT population (Excluding 16 patients from CRTN 14158). Baseline CD4 Counts (cells/mm3) and Plasma RNA (log_{10} copies/mL).**

Treatment	ddC	SAQ 600mg	SAQ 600mg+ddC
Baseline CD4			
N	134	151	137
Mean	175	158	186
Median	176	159	156
Range	5-411	24-337	10-381
Baseline HIV-RNA			
N	120	130	123
Mean	5.2	5.2	5.1
Median	5.2	5.3	5.3
Range	2.7-6.5	3.0-6.2	3.0-6.1

Source: CANDA SR for NV14256; W144954, pg. 36 and 159.

8.1.2.4.3 Activity/efficacy endpoint outcomes

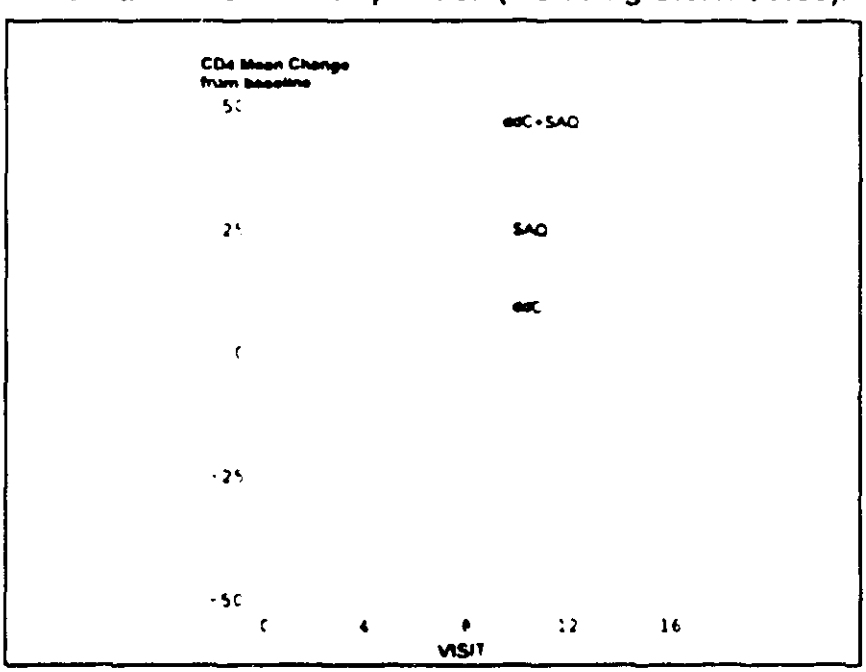
Table 28 shows mean/median CD4 changes from baseline for each treatment arm. Only the combination group, ddC+SAQ, had mean CD4 increases from baseline that were maintained over the 16 week surrogate study period. The mean change in CD4 was below baseline by week 16 for the ddC arm and only 5 cells above baseline for the SAQ arm. Figure 3 is a plot of the mean changes from baseline over the 16 week surrogate analysis period.

Table 28. NV14256: FDA ITT population. CD4 Counts - Changes (mean/median) from Baseline (cells/mm3) up to Week 16.

VISIT/ WEEK	4	8	12	16
ddC				
N	132	127	118	117
Mean	14	13	0.4	-11
Std Dev	61	71	78	53
Median	7	6	-10	-8
SAQ 600mg				
N	150	137	136	131
Mean	23	28	14	5
Std Dev	67	66	67	64
Median	11	15	2	-8
SAQ 600mg+ddC				
N	134	129	124	119
Mean	35	45	41	45
Std Dev	61	75	83	135
Median	29	37	21	26

Source: CANDA SR for NV14256, W144'954, pg 155

Figure 3. CD4 Counts - Mean Changes from Baseline (cells/mm3) up to Week 16. FDA Intent to Treat Population (Including CRTN 14158).



We used an Analysis of Variance (ANOVA) to compare changes in absolute CD4 cell counts and RNA (DAVG-16 metric). The factors included in the analyses models were treatments, study center (in this case, region) and the protocol-specified stratification factor, pre-entry CD4 category (<100 cells/mm^3 and ≥ 100 cells/mm^3).

Table 29 shows the mean CD4 change from baseline averaged over 16 weeks as calculated by the DAVG-16 method. Included are 95% confidence intervals (Scheffe's) around the mean. The combination treatment produced the largest increase in CD4 from baseline followed by SAQ monotherapy. The lower confidence interval for ddC monotherapy was negative, indicating decreases in CD4 from baseline.

Table 29. NV14256: FDA ITT population. Summary of CD4 changes from baseline (DAVG-16).

TREATMENT	N	MEAN	95% C.I.*
SAQ	158	16	5, 27
ddC	140	6	-5, 17
ddC+SAQ	141	34	22, 45

Source Kazem Kazempour, SAS applications
Scheffe's confidence intervals

An FDA analysis showed that, overall, the three treatments were statistically different in their effect on mean CD4 changes from baseline. Region or pre-entry CD4 strata were not significantly different with respect to their association with mean CD4 changes (See table 30).

Table 30. Overall comparisons.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	2	53777 70	26888 85	11 63	0.0001
CD4 strata	1	1463 23	1463 23	0 63	0.4267
Region	13	21208 61	1631 43	0 71	0.7580

Source Kazem Kazempour, SAS applications

Table 31 shows pairwise comparisons between treatment groups for mean CD4 change from baseline (DAVG-16) The combination treatment was statistically superior to both SAQ and ddC monotherapy for increases in CD4 from baseline At present it is not known whether these differences in CD4 changes will be clinically relevant There was a trend toward greater CD4 increases in the SAQ group compared to the ddC group.

Table 31. NV14256: FDA ITT analysis of pairwise comparisons for mean CD4 changes from baseline (DAVG-16)

TREATMENT	Difference in Means	95% C.I.**	Pr>F
ddC+SAQ vs. SAQ	18	4, 31	0.0001
ddC+SAQ vs. ddC	28	14, 42	0.0021
SAQ vs. ddC	10	-4, 24	0.0722

Source: Kazem Kazempour, SAS applications
**Scheffe's confidence intervals

HIV-RNA

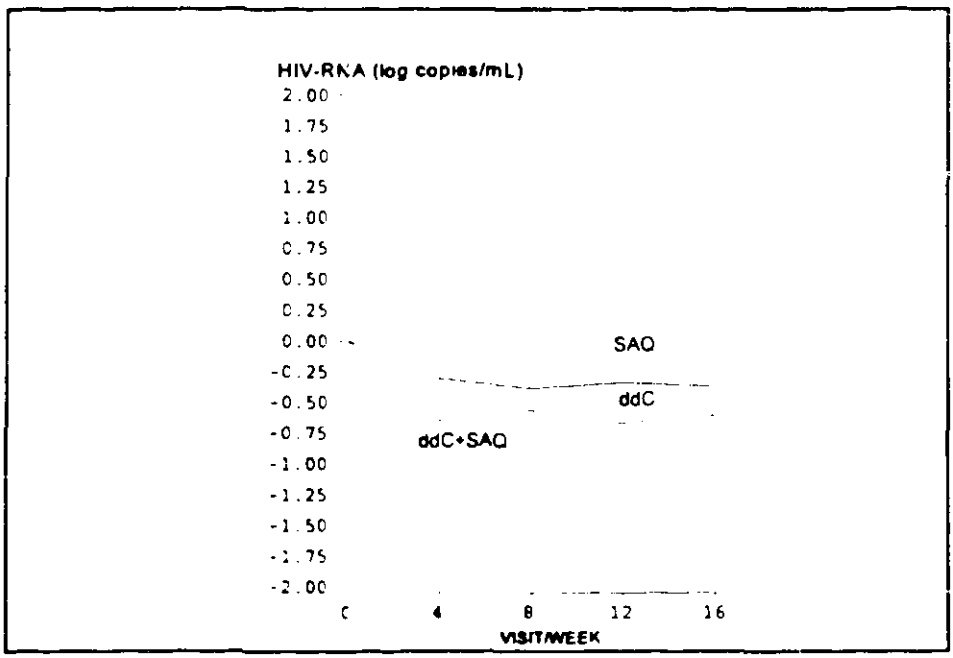
Mean/median changes in HIV-RNA as measured by PCR (Roche method) are displayed in Table 32. The combination treatment produced the largest reductions in HIV-RNA at each visit. As was observed for CD4 changes, the combination treatment's effect on RNA was maintained over the 16 week period. The effect of SAQ monotherapy was small and diminished over the 16 week period. Figure 4 plots the mean changes in RNA over 16 weeks.

Table 32. Log_{10} HIV-RNA (PCR) - Change (mean/median) from baseline (copies/mL) up to Week 16. FDA ITT Population (including center 14158)

VISIT/WEEK	4	8	12	16
ddC				
N	108	92	93	87
Mean	-0.32	-0.40	-0.35	-0.38
Std Dev	0.44	0.51	0.46	0.50
Median	-0.22	-0.33	-0.29	-0.32
SAQ 600mg				
N	109	104	98	101
Mean	-0.23	-0.17	-0.17	-0.08
Std Dev	0.47	0.39	0.43	0.40
Median	-0.12	-0.10	-0.13	-0.03
SAQ 600mg+ddC				
N	111	101	100	96
Mean	-0.66	-0.57	-0.68	-0.61
Std Dev	0.65	0.62	0.65	0.72
Median	-0.57	-0.54	-0.61	-0.56

Source: CANDA SR for NV14256 W144954.pg 1

Figure 4. NV14256 FDA ITT Population. Mean change in HIV-RNA (log copies/mL) from baseline.



Source Kazem Kazempour, SAS applications

Table 33 shows the mean RNA changes (log10) averaged over 16 weeks (using the DAVG metric) for each treatment arm In the FDA analysis of RNA changes, 367 subjects are included in the intent to treat population. It should be noted that fewer patients were included in the analysis of RNA changes than for the analysis of CD4 changes Reasons for missing RNA data included: no post-baseline samples drawn, no baseline samples drawn, and inappropriate processing, storage, or shipment of samples

Table 33. NV14256: FDA ITT analysis. Mean changes in HIV- RNA (copies/mL) over a 16 Week Period, DAVG-16.

TREATMENT	N	MEAN	95% C.I.*
SAQ	127	-0.1	-0.2, 0.0
ddC	118	-0.3	-0.4, -0.2
ddC+SAQ	122	-0.5	-0.6, -0.4

Source Kazem Kazempour, SAS applications
Scheffe's confidence intervals

Overall, in our analysis, there was a statistically significant difference among

the three treatment arms for changes in HIV-RNA. There was also a statistically significant effect between baseline CD4 strata (<100, ≥100 cells/mm^3) and change in RNA. Patients with screening CD4 counts > 100 cells/mm^3 had statistically greater reductions in log transformed RNA copies/mL RNA than those with < 100 CD4 cells/mm^3 at baseline. There were no statistically significant differences between regions with respect to change in RNA over 16 weeks (See Table 34).

Table 34. FDA analysis of log transformed RNA changes by protocol-specified independent variables.

Variable	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	2	8.93	4.46	31.38	0.0001
CD4 strata	1	1.04	1.04	7.28	0.0073
Region	13	2.71	0.21	1.47	0.1278

Source: Kazem Kazempour, SAS applications

In pairwise comparisons, the combination (ddC+SAQ) produced significantly greater RNA reductions (log transformed copies/mL) than either monotherapy. (See Table, 35 for difference in means, 95% confidence intervals and p-values) In addition the ddC monotherapy produced significantly greater RNA reductions than the SAQ monotherapy. The clinical significance of these differences in RNA reductions are not known.

Table 35. Pairwise Comparisons of treatment groups(Scheffe's test).

TREATMENT	Difference in Means	95% C.I.**	Pr>F
Comb. vs SAQ	-0.39	-0.51, -0.28	0.0001
Comb. vs ddC	-0.23	-0.35, -0.11	0.0001
ddC vs. SAQ	-0.16	-0.28, -0.05	0.0011

Source: Kazem Kazempour, SAS applications
**Scheffe's confidence intervals

Additional Analyses

In the surrogate cohort, 72/423 patients (17%) had no baseline or post-baseline RNA data. This was a higher percentage of missing data than observed in any of the other studies in the clinical program. To investigate if these missing data could have had an impact on the overall conclusions, we compared CD4 changes in all patients (n=439) with those patients who had both CD4 and RNA specimens (n=367) Table 36 shows the mean changes in CD4 from baseline in those patients who had both CD4 and HIV-RNA samples. Results were similar for these two groups. The conclusions are the

same.

Table 36. FDA analysis of CD4 change - DAVG-16 (cells/mm³) for patients who had both RNA and CD4 data available (n=367).

TREATMENT	N	MEAN	95% C.I.*
SAQ	127	14	3, 26
ddC	118	4	-8, 16
ddC+SAQ	122	36	24, 48

Source: Kazem Kazempour, SAS applications.
Scheffe's confidence intervals

Clinical endpoints

This study is ongoing and remains blinded with respect to clinical endpoints. However, Appendix II lists clinical endpoints occurring in the surrogate cohort. The AIDS-defining clinical endpoints listed in this table were not validated by the clinical events committee and do not include all AIDS defining events in this trial.

8.1.2.4.4 Safety comparisons

Safety comparisons include data up to June 1, 1995, the date of closure for the updated safety data base. The duration of treatment for patients in the surrogate analysis cohort is shown in Table 37. Median duration of exposure to study treatment was approximately 42 weeks for this cohort by the above closing date.

Table 37. NV14256: Duration of Exposure to Trial Treatment (ITT population) by safety data base closure of June 1, 1995.

Treatment	ddC	SAQ	SAQ +ddC
Total Patients	140	158	141
Duration (weeks)			
0 - 4	140	158	141
> 4 - 8	135	153	135
> 8 - 12	131	145	132
>12 - 16	125	140	125
>16 - 20	120	136	123
>20 - 24	114	131	116
>24 - 28	98	117	104
>32 - 36	90	109	102
>36 - 40	77	103	97
>40 - 44	70	91	88
>44 - 48	52	55	68

Source CANDA SR for NV14256, W144954, pg 34

Table 38 lists the number of patients with at least one adverse event and the number (percentage) of patients experiencing specific types of adverse events. The events shown are include all intensities (mild, moderate, severe, life threatening), but exclude events that the investigator considered to be unrelated to study treatment (usually events that were attributed to the underlying disease). Events are listed if they occur with a frequency of at least 3% in any treatment arm.

Diarrhea appeared to be more frequent among patients randomized to a SAQ containing arm, occurring in 16% of patients on SAQ and 5% of patients on ddC. All of the cases of diarrhea were either mild or moderate; there were no cases of severe or life-threatening diarrhea, except one occurring in the ddC group. Toxicities known to be associated with ddC, specifically peripheral neuropathy and buccal mucosal ulceration, were more frequent among patients randomized to ddC containing arms. Peripheral neuropathy is associated with HIV infection as well as drugs such as ddC. HIV infection may explain some of the cases of peripheral neuropathy seen among patients taking SAQ, since the neuropathy among these patients was not severe. For example, 4% of patients on SAQ monotherapy had peripheral neuropathy of any severity. However when considering only moderate or worse neuropathy, the frequency of peripheral neuropathy among patients on the SAQ arm was 0%.

Table 38. Number (percentage) of patients with AEs of all intensities excluding unrelated. Listed if at least 3% in any treatment group Safety update, data up to June 1, 1995

ADVERSE EVENT	ddC *N=145*	SAQ *N=159*	ddC + SAQ *N=147*
ALL BODY SYSTEMS			
Total Patients with at least one AE	76 (52)	75 (47)	78 (53)
Total number Aes	205	161	171
GASTROINTESTINAL SYSTEM			
Buccal mucosal ulceration	22 (15)	10 (6)	15 (10)
nausea	10 (7)	7 (4)	5 (3)
abdominal discomfort	9 (6)	10 (6)	6 (4)
abdominal pain	3 (2)	5 (3)	2 (1)
diarrhea	7 (5)	25 (16)	20 (14)
CENTRAL AND PERIPHERAL NERVOUS SYSTEM			
penpheral neuropathy	19 (13)	7 (4)	13 (9)
numbness extremities	10 (7)	4 (3)	9 (6)
paraesthesia	7 (5)	2 (1)	6 (4)
headache	12 (8)	7 (4)	8 (5)
dizziness	4 (3)	2 (1)	2 (1)
GENERAL			
asthenia	12 (8)	7 (4)	6 (4)
fever	4 (3)	5 (3)	2 (1)
SKIN AND APPENDAGES			
rash	9 (6)	7 (4)	7 (5)
MUSCULOSKELETAL SYSTEM			
myalgia	6 (4)	3 (2)	3 (2)
musculoskeletal pain	4 (3)	2 (1)	3 (2)
arthralgia	4 (3)	1 (<1)	2 (1)
PSYCHIATRIC			
insomnia	6 (4)	1 (<1)	4 (3)

Source CANDA SR for NV14256 W144954 Table 17, pg 52-62 and NDA Safety update pages 48-59.

Table 39 lists the number (percentage) of patients experiencing an adverse

event according to the severity of the event. A smaller percentage of patients on SAQ monotherapy (5%) experienced severe or life-threatening events compared to those receiving ddC monotherapy (15%) or ddC + SAQ (10%).

Table 39. NV14256: Number of patients experiencing an adverse event (excluding unrelated) by severity.

TREATMENT	TOTAL	MILD	MODERATE	SEVERE	LIFE-THREAT.	UNK
ddC *N=145*	76 (52)	50 (35)	40 (28)	21 (15)	1 (<1)	-
SAQ *N=159*	75 (47)	48 (30)	36 (23)	7 (4)	2 (1)	1 (<1)
ddC+SAQ *N=147*	78 (53)	50 (34)	34 (23)	15 (10)	1 (<1)	-

Source: NDA Safety Update Table 8, pages 48-59

Deaths

Roche reported that 12 patients in the surrogate analysis cohort died during the study. Seven patients died prior to the clinical cut-off date (Jan. 1995) and 5 additional patients died by the updated safety date closure (June 1, 1995). There were five deaths each on the SAQ and ddC arms and 2 deaths on the combination arms. All but one of these deaths were attributed to complications of AIDS (such as opportunistic infections, bacterial pneumonias, dementia and inanition) and were considered by the investigator to be unrelated to study medication. The exception was a patient randomized to ddC who died of congestive heart failure secondary to HIV cardiomyopathy. This was considered to be remotely related to ddC treatment.

Comment:
From what has been reported on the case report forms, it appears that all of the 12 deaths are most likely AIDS related, as Roche has reported. However, Roche submitted 13 case report forms for patients who had died before the safety closure dates. this additional death (occuring on 6/1/94) was judged by the investigator to be unrelated to treatment. Cause of death on the death certificate, according to the case report form, was cardiorespiratory arrest, kidney failure and AIDS. Several weeks prior to this patient's death the patient suffered a myocardial infarction, pulmonary edema, and presumptive aspiration pneumonia.

Three deaths have occurred among patients not included in the surrogate cohort by the time of the last data base closure. These deaths were all considered unrelated to treatment. Causes of death in these three patients were listed as AIDS, stretococcal pneumonia/Mycobacterial bacteremia, and possible malaria superimposed on progressive HIV infection. Since the trial is

ongoing, these patients have not been unblinded for endpoints.

Serious Adverse Events
In the surrogate analysis population, 57 patients had events that were deemed serious. Several had more than one serious event. There were two cases of pancreatitis (one receiving ddC and one receiving ddC+SAQ). There were no cases of severe hepatomegaly with steatosis or unexplained lactic acidosis.

Eight patients receiving SAQ or SAQ+ddC had serious adverse events considered to be at least remotely related to treatment. These events were:

1) ataxia (remote)
2) severe bronchitis, also moderate hemoptysis and re-hospitalization due to fever and disorientation; a diagnosis of severe Klebsiella pneumonia was made. The investigator considered that these adverse events were remotely related to treatment
3) pseudomembranous colitis (remote)
4) pancreatitis (possible) This patient was on ddC+SAQ
5) psychotic disorder, seizures (remote)
6) depression (possible)
7) hepatitis A (remote)
8) pneumonia

Comment:
Case number 7, hepatitis A should be considered unrelated.

Among the patients (497) that were not included in the surrogate analysis cohort, there were 33 serious adverse events in 29 patients. Five of these events were considered to be at least remotely related to treatment. These were pneumonia-remote, rash/liver enzyme disorder-possible, suicide attempt-remote, convulsions-remote. These patients are still blinded.

Adverse Events Leading to Premature Withdrawal from Treatment
A total of 79 patients in the surrogate analysis cohort withdrew from treatment due to protocol treatment or non-protocol treatment toxicity. Of those patients, there were 35 withdrawals in the ddC group, 22 in the SAQ group and 22 in the SAQ + ddC group (see Table 40). Protocol treatment toxicities are those that required dose adjustment according to the protocol adverse event management section. In general these were severe of life-threatening toxicities (ACTG grades 3 or 4).

Table 40. Number of Patients Withdrawing due to Protocol or Non-Protocol Treatment Toxicity.

	No. of patients withdrawing from treatment prematurely		
	ddC n = 145	SAQ n = 159	SAQ + ddC n= 147
Protocol treatment toxicity	25	9	17
Non-Protocol treatment toxicity	10	13	5
Total	35	22	22

Source: CANDA SR for NV14256, W144'954, table 20, pg. 70

Most of the protocol-specified treatment discontinuations, for protocol-specified treatment toxicities, were for peripheral neuropathy, 13/25 on ddC, 14/17 on ddC+SAQ, 2/9 on SAQ. In addition, 5/25 discontinuations among patients randomized to ddC were due to buccal mucosal ulceration. One patient each, on SAQ and SAQ+ddC discontinued treatment secondary to pancreatitis, and another patient on the combination discontinued treatment secondary to hyperamylasemia without clinical pancreatitis. One patient on ddC and two on SAQ monotherapy discontinued treatment after developing grade 3 or 4 liver enzyme abnormalities. One patient discontinued ddC because of elevated liver function tests that were attributed to alcohol use.

Twenty-four patients not included in the surrogate cohort discontinued drug for toxicity (16 for protocol treatment toxicity and 8 for non-protocol treatment toxicity)

Laboratory Abnormalities

Table 41 shows the percentage of patients with marked laboratory abnormalities. There does not appear to be any imbalance among treatment arms for marked laboratory abnormalities. Although a few patients on the SAQ arm discontinued drug due to elevation of transaminases, less than 1% of individuals had marked elevations of AST or ALT compared to approximately 3% of patients on ddC monotherapy.

Table 41. Summary of Marked Laboratory Abnormalities

Marked Laboratory Abnormalities	Percentage of Patients with Marked Laboratory Abnormalities		
	ddC n=145	SAQ n=159	ddC + SAQ n=147
High CPK	6	4	7
Low Glucose	4	5	5
High ALT	3	<1	<1
High AST	3	<1	<1
High Potassium	2	<1	<1
High serum amylase	<1	<1	2
High glucose	0	<1	<1
High calcium	<1	0	0
High bilirubin	0	1	0
Hemoglobin (low)	1	<1	0
Low platelets	0	0	<1

Source NDA Safety update Table 10 pg 71

Summary of Safety data from the ddC + SAQ 200 mg tid treatment arm
A total of 108 patients were randomized to the ddC + SAQ treatment arm. The overall pattern of adverse events was very similar to that seen in the ddC and ddC+SAQ 600 tid mg arms of the surrogate analysis cohort . The adverse events most frequently reported were diarrhea, asthenia, rash, peripheral neuropathy, nausea and headache One death occurred among those randomized to the ddC + saquinavir 200 mg tid treatment group. The primary cause of death was attributed to HIV end stage renal disease, and was considered unrelated to study treatment

Five patients in the SAQ 200 mg + ddC treatment group had a total of 7 serious adverse Two patients had serious adverse events considered related to trial treatment One patient was hospitalized for severe hemolytic anemia and moderate elevation of CPK Both were considered to be remotely related to study treatment The second patient developed a highly malignant immunoblastic B cell lymphoma that the investigator considered to be remotely related to treatment

A total of 6 patients in the SAQ 200 mg + ddC treatment group withdrew from treatment before the treatment arm was discontinued, 4 of these were due to protocol treatment toxicity These toxicities included a severe rash (probably

related), severe hepatosplenomegaly and severe liver enzyme disorder (possibly related), severe back and abdominal pain (remotely related), moderate diarrhea and recurrence of moderate buccal mucosa ulceration (probably related).

8.1.2.3 Reviewer's Conclusions

Activity

This study demonstrated superior antiviral activity of ddC+SAQ compared to a ddC monotherapy control. Both CD4 and RNA changes for the combination were statistically significant compared to ddC monotherapy and SAQ monotherapy. The mean CD4 increase from baseline for ddC+SAQ averaged over 16 weeks was 34 cells/mm^3. The mean plasma HIV-RNA reduction averaged over 16 weeks was 0.6 log_{10} copies/mL. The clinical significance of these changes in markers of antiviral activity is not known. The changes in markers appeared to be maintained over the 16 week period, although approximately one-quarter of the participants had discontinued study medications by this time point.

Interpreting the relative activity of SAQ monotherapy compared to ddC is more difficult. For this comparison there is a discordance in the results for the two primary markers. For CD4 changes, SAQ produced greater mean increases than ddC, although not achieving statistical significance. For HIV-RNA reduction ddC was statistically superior to SAQ. These results make it difficult to conclude which drug is more active.

Safety

Compared to ddC, SAQ appears to have fewer adverse effects. Adverse events occurring in greater than 3% of individuals were diarrhea, abdominal discomfort, abdominal pain, nausea, buccal mucosal ulceration, peripheral neuropathy, numbness of extremities, headache, rash, asthenia, fever. The only events that occurred more frequently on the SAQ arm than on ddC was diarrhea (16% vs 5%, respectively) and abdominal pain (3% vs 2%, respectively). When combined with ddC, SAQ did not alter the frequency or severity of events known to be associated with ddC alone. In this study there were no specific laboratory abnormalities observed with greater frequency on SAQ than on ddC. Although a few patients on the SAQ arm discontinued drug due to elevation of transaminases, less than 1% of individuals had marked elevations of AST or ALT compared to approximately 3% of patients on ddC monotherapy.

8.1.3 Protocol V-13330 (Italy)

"A randomized, double-blind study to investigate the antiviral activity, tolerability and pharmacokinetics of varying doses of oral Ro-31-8959 in combination with a fixed dose of zidovudine administered to previously untreated symptomatic HIV-infected individuals."

Comment:
It should be noted that this trial and the following two European trials were already enrolled and ongoing at the time of Roche's initial IND submission in the U.S.; therefore, these protocol designs were not reviewed by the FDA.

8.1.3.1 Protocol

8.1.3.1.1 Objectives

The primary objectives as written in the protocol are: "To investigate the dose response relationship as measured by the antiviral activity and tolerability of varying doses of SAQ in combination with a fixed dose of ZDV in symptomatic HIV-infected patients To investigate the antiviral activity of combination SAQ + ZDV therapy compared to the antiviral activity of the single agents."

8.1.3.1.2 Design

This was a 16 week randomized, double blind, phase 1/2 study with optional monthly extensions Subjects were to be randomized to one of the following treatment regimens

- ZDV 200 mg tid + SAQ placebo
- ZDV 200 mg tid + SAQ 75 mg tid (Combo-75)
- ZDV 200 mg tid + SAQ 200 mg tid (Comb-200)
- ZDV 200 mg tid + SAQ 600 mg tid (Combo 600)
- ZDV 200 mg tid placebo + SAQ 600 mg tid

SAQ was administered within 30 minutes after a meal.

Stratification and blinding

Patients were stratified by p24 antigenemia (< 50pg/mL, ≥ 50 pg/mL). Blinding of the dosage was accomplished by use of matching SAQ and ZDV placebo capsules During the double-blind extension phase subjects remained on the same blinded treatment they received during the first 16 weeks of treatment

Procedures

The full pharmacokinetic profile of SAQ and ZDV was to be assessed on days 7 and 42 Samples for drug analysis were also taken on weeks 8, 12, and 16.

Activity laboratories were to be obtained at two screening visits and on day 1, weeks 1, 2, 3, 4 and monthly thereafter. The final study report uses the median of screenings and day 1 to calculate a baseline for CD4 counts.

8.1.3.1.3 Study Population

Inclusion criteria (summarized)

The goal of the protocol was to recruit 75 evaluable study subjects (15 per treatment arm) from 5 clinic sites in Italy. To be eligible, study subjects were required to be age 18-65, CDC HIV class III or IV (1987 definition) with CD4 counts ≤ 300 cells/mm^3. Females were included.

Comment:

This study and study 0-13328 are the only clinical protocols in this NDA that included individuals with baseline CD4 counts < 50 cells/mm^3.

Exclusion Criteria (from CANDA FSR for V-13330, W'144'918; section 2.4.3 and 2.44 pages 13 and 14)

- neoplasms other than cutaneous Kaposi's sarcoma or basal cell carcinoma
- previous or current history of chronic systemic disorder requiring treatment in the preceding 12 months
- intolerable diarrhea of duration greater than 15 days and requiring treatment within 30 days prior to starting therapy
- pregnancy or lactation
- current history of serious oral or parenteral drug abuse
- HIV-encephalopathy (HIV-dementia, AIDS-dementia, sub-acute encephalitis due to HIV, and multi-focal leucoencephalopathy)
- history of psychiatric disorders including non-reactive depression
- acute serious opportunistic infections requiring immediate treatment, and including tuberculosis, CMV, cerebral toxoplasmosis and Pneumocystis carinii pneumonia
- CMV or an atypical mycobacterial infection requiring secondary chemoprophylaxis
- abnormal ECG of clinical relevance
- proven Idiopathic Thrombocytopenic Purpura
- previous or current treatment with ZDV, ddC, ddI or other experimental anti-retrovirals or immunomodulators
- previous treatment with interferons within the last 12 months
- systemic treatment with corticosteroids within 30 days of screening or starting therapy
- treatment with any investigational agent, antineoplastic agent or radiotherapy (excluding local skin radiotherapy) within 12 weeks of screening or starting therapy. Subjects were to be excluded for previous or current treatment with any antiretroviral. Concomitant use of ganciclovir or foscarnet was also prohibited

8.1.3.1.4 Endpoints

At the time the protocol was written, the primary activity parameters to be assessed were absolute CD4 count, CD4 percent and HIV p24 antigen (Coulter test kit). Secondary activity parameters included serum neopterin, serum β-2 microglobulin, quantitative PCR using plasma (RNA) and mononuclear cells (DNA), and plasma and mononuclear cell associated quantitative viremia

Comment:
The emphasis placed on the activity parameters in the final study report differs from those outlined in the protocol reflecting changes in scientific opinion regarding the relative importance of these markers since the design of the trial. Specifically, in the extension phase report, more emphasis is placed on the RNA-PCR measurements rather than p24.

In the protocol, demonstration of antiviral activity was defined by fulfilling one or more of the following criteria:

- A CD4 count rise of ≥ 75 cells, confirmed by a second evaluation at least one month later.
- A ≥70% reduction in p24 antigen, confirmed by a second evaluation at least one month later.
- A ten-fold reduction in either quantitative viremia or quantitative RNA/DNA PCR, confirmed by a second evaluation at least one month later.

Comment:
This division has preferred analyzing primary activity variables, (i.e., CD4 counts) as continuous data rather than conversion to nominal response variables. Changes are evaluated by averaging mean changes over time. This preserves statistical power and obviates the need for defining arbitrary response criteria. DAVG over 16 or 24 weeks has been FDA's preferred method for analyzing changes in CD4 based on research by Drs. Stella Machado and Kazem Kazempour. In their final study reports, Roche analyzes activity parameters by this method in addition to looking at response categories.

8.1.3.1.5 Statistical considerations

Analyses
The protocol states that all patients will be included in the ITT analysis.

Comment:
In actuality, the ITT population included all those who were randomized and received drug. Two patients who did not receive drug were excluded.

Sample size
The sample size of 75 subjects was chosen according to "practical limitations." The protocol states that 15 subjects per arm should be sufficient to detect a dose response trend across the four SAQ-containing arms when the response rates between the worst and best combination differ by 50%. (type 1 error is 0.025, reduced for interim an look).

Comment:
A protocol amendment deleted the interim analysis, therefore, the trend analysis for dose response used a type 1 error of 5%.

Safety data analysis
The time window for inclusion of safety data (adverse events and laboratories) was up to 4 days following the final dose of trial treatment, except for reporting of deaths which was up to 28 days following the final dose.

8.1.3.2 Results

The 16 week trial period was conducted from February, 1992 - February, 1993. The double blind extension period lasted from February, 1993 - March, 1993

8.1.3.2.1 Patient Disposition

Of 94 patients enrolled and randomized to study treatment, 92 received treatment and 80 completed the study. Table 42 shows the number of patients completing 16 weeks of therapy and reasons for withdrawal for those subjects who did not complete 16 weeks.

Table 42. V-13330 (Italy): Disposition of patients randomized, first 16 weeks.

Treatment Group	Randomized	Completed 16 weeks	Reasons for withdrawal
SAQ 600 mg	20	17	1 never received drug 2 AE/intercurrent illness
ZDV 200 mg	18	17	1 never received drug
Combo-75	18	14	1 pregnancy 3 AE/intercurrent illness
Combo-200	18	15	1 non-cooperation 1 withdrew consent 1 AE/intercurrent illness
Combo-600	20	17	2 AE/intercurrent illness 1 Concomitant medication
Total	94	80	14

Source: CANDA FSR for V-13330, W-144-918, p. 29

Double-blind extension phase
Table 43 shows the duration of study treatment through the double blind extension phase. Eighty subjects entered the double-blind extension phase and 49 subjects completed it. The number of subjects continuing per treatment group past week 40 was small. There was a lower drop-out rate in the Combo-600 group as compared to other groups; 13/20 patients

completed 40-44 weeks of treatment. At least one patient in each of the 5 treatment groups received more than 52 weeks of treatment before the end of the double-blind extension phase; 3 patients received 56 weeks of treatment.

Table 43. V-13330 (Italy): Duration (weeks) of Exposure to Trial Treatment.

TREATMENT	SAQ 600mg + Placebo	Placebo + ZDV	SAQ 75mg + ZDV	SAQ 200mg + ZDV	SAQ 600mg + ZDV
Treatment Duration (weeks)					
0 - 4	19	17	18	18	20
>4 - 8	18	17	16	17	19
>8 - 12	18	17	15	17	18
>12 - 16	18	17	14	16	17
>16 - 20	17	14	12	14	16
>20 - 24	15	14	12	13	16
>24 - 28	14	12	12	12	16
>28 - 32	13	12	11	12	15
>32 - 36	12	10	10	12	15
>36 - 40	12	10	10	10	15
>40 - 44	10	8	6	7	13
>44 - 48	4	4	4	3	9
>48 - 52	3	3	3	2	4
>52 - 56	1	1	2	2	2
>56 - 60	0	0	0	0	0

Source: CANDA FSR for V-13330, W144'933, p.56

8.1.2.5.2 Demographics and baseline distribution

This study differed from many U.S. clinical trials in that intravenous drug use was the most common means of HIV transmission in 4/5 treatment arms. In the fifth arm heterosexual transmission was listed as the most common route of transmission. Thirty of the 92 individuals in the safety/ITT population were females.

Table 44 shows mean/median baseline CD4 counts by treatment group. Median baseline CD4 counts across all treatment arms ranged from 160 to 257 cells/mm^3. At study center Bari, which enrolled 21 subjects (distributed between the 5 treatment arms), there were several problems with measurements of lymphocytes subsets. Due to these problems, Roche excluded the 21 patients enrolled at Bari in their analyses of CD4. The decision to exclude these individuals was made months before the unblinding of treatment groups. These problems included:

- inadequate quality control
- technical fault leading to no processing of samples between 4/24/92 and 5/14/92
- probable operator error in dilution of OKT4 FITC monoclonal antibody between 7/3/92 and 7/19/92 (internal check indicated one value was 37% too high)

Comment:
FDA analysis includes patients from the Bari center. There were nine patients who had one of their week 0-16 CD4 counts measured during the two-week time period that was suspect for unreliable CD4 measurements. These patients were distributed among treatment groups as follows:

2 on ZDV
2 on SAQ
1 on ZDV/SAQ 75
1 on ZDV/SAQ 200
3 on ZDV/SAQ 600

Table 44. V-13330 (Italy): Baseline CD4 Counts (cells/mm3) - FDA, ITT Population

TREATMENT	SAQ 600mg Placebo	Placebo ZDV	SAQ 75mg ZDV	SAQ 200mg ZDV	SAQ 600mg ZDV
INCLUDING BARI					
N	19	17	18	18	20
Mean	172	168	217	152	173
Median	198	172	235	126	172
EXCLUDING BARI					
N	15	13	14	14	15
Mean	180	155	220	155	168
Median	210	156	244	155	157

Source: CANDA FSR for V-13330, W144'918 Table 7 and 8, pages 40, 41.

8.2.2.6.3 Activity endpoints

For CD4

Table 45 shows the mean/median CD4 changes from baseline at each visit for the five treatment groups over the 16 week study period. This data includes the Bari center. CD4 increases in this trial were greater than that seen in the other European trial. This trial included treatment naive individuals and SAQ in combination with ZDV

TABLE 45. V-13330 (Italy): Mean/median changes in CD4 Counts from baseline at each visit for five treatment arms.

VISIT/ WEEK	1	2	3	4	8	12	16
SAQ 600mg							
N	19	19	18	16	18	18	18
Mean	37	70	42	37	36	49	44
Std Dev	45	60	52	47	72	73	106
Median	47	65	32	32	18	64	8
ZDV							
N	16	16	16	15	13	17	16
Mean	52	49	60	48	37	25	18
Std Dev	81	90	96	81	60	86	78
Median	30	23	32	25	50	10	6
SAQ 075mg-ZDV							
N	15	17	18	14	16	14	13
Mean	54	20	37	44	78	28	22
Std Dev	54	43	67	52	85	75	67
Median	59	22	32	39	57	11	21
SAQ 200mg-ZDV							
N	16	15	16	17	18	15	13
Mean	60	69	56	59	46	53	29
Std Dev	77	53	72	75	71	68	65
Median	37	77	51	61	28	47	14
SAQ 600mg-ZDV							
N	18	19	19	18	17	18	18
Mean	32	69	82	86	62	96	101
Std Dev	43	75	83	95	71	114	116
Median	17	55	61	57	50	63	69

Source generated from SAS applications of CANDA

Table 46 shows mean change in CD4 count from baseline averaged over 16 weeks for the five treatment groups

Table 46. V-13330 (Italy): FDA ITT Population. Mean changes in CD4 from baseline (cells/mm^3) DAVG-16.

TREATMENT	N	MEAN	95% C.I.*
SAQ	19	44	6, 83
ZDV	17	34	-7, 74
SAQ 75 mg + ZDV	18	36	4, 75
SAQ 200 mg + ZDV	18	43	3, 82
SAQ 600 mg + ZDV	20	80	42, 117

Source: Kazem Kazempour, SAS applications
Scheffe's confidence intervals

Overall, there was a statistical trend (p=0.059) for a difference between treatment arms with respect to change in CD4 from baseline averaged over 16 weeks. No pairwise comparisons achieved statistical significance however ZDV+SAQ 600 mg showed a trend toward superior CD4 increases from baseline compared to the control ZDV. There was a statistical difference between centers with respect to CD4 change (See table 47)

TABLE 47. Overall Compansons of CD4 analysis (DAVG-16).

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	22831 42	5707 85	2 37	0 0592
Center	4	73210 85	18302 71	7 59	0 0001

Source Kazem Kazempour, SAS applicabons

We analyzed the CD4 data excluding the nine patients who had at least one 0-16 week CD4 value measured during the time period that was suspect for unreliable measurements A DAVG-16 analysis (using Roche's method for calculating DAVG-16, generated from the CANDA) excluding these patients resulted in lower means for all treatment groups but the conclusions remain the same The ZDV+SAQ 600 mg dose produced the highest mean increases in CD4 counts averaged over 16 weeks Neither analysis achieved statistical significance Table 48 compares mean CD4 changes from baseline including and excluding these nine individuals

Table 48. Comparison of DAVG-16 analyses of CD4 including and excluding nine patients.

Treatment	SAQ 600mg	ZDV	SAQ 075mg/ZDV	SAQ200mg/ZDV	SAQ 600mg/ZDV
ALL PATIENTS					
n	19	17	18	18	20
Mean	43	31	39	46	68
EXCLUDING 9					
n	17	15	17	17	17
Mean	32	22	33	43	67

Generated using Roche CANDA

RNA PCR

The RNA-PCR specimens were processed after the study was unblinded, however those performing the evaluations were kept blinded to treatment identities. Table 49 shows the baseline HIV-RNA measurements (log transformed copies/mL)

Table 49. V-13330 (Italy): FDA ITT population. Baseline HIV-RNA (log copies/mL).

TREATMENT	SAQ 600mg Placebo	Placebo ZDV	SAQ 75mg ZDV	SAQ 200mg ZDV	SAQ 600mg ZDV
Baseline					
N	19	17	18	18	20
Mean	5.2	5.2	5.2	5.5	5.3
Median	5.2	5.3	5.3	5.5	5.3

Source CANDA FSR for V-13330, w144'933, pg 47

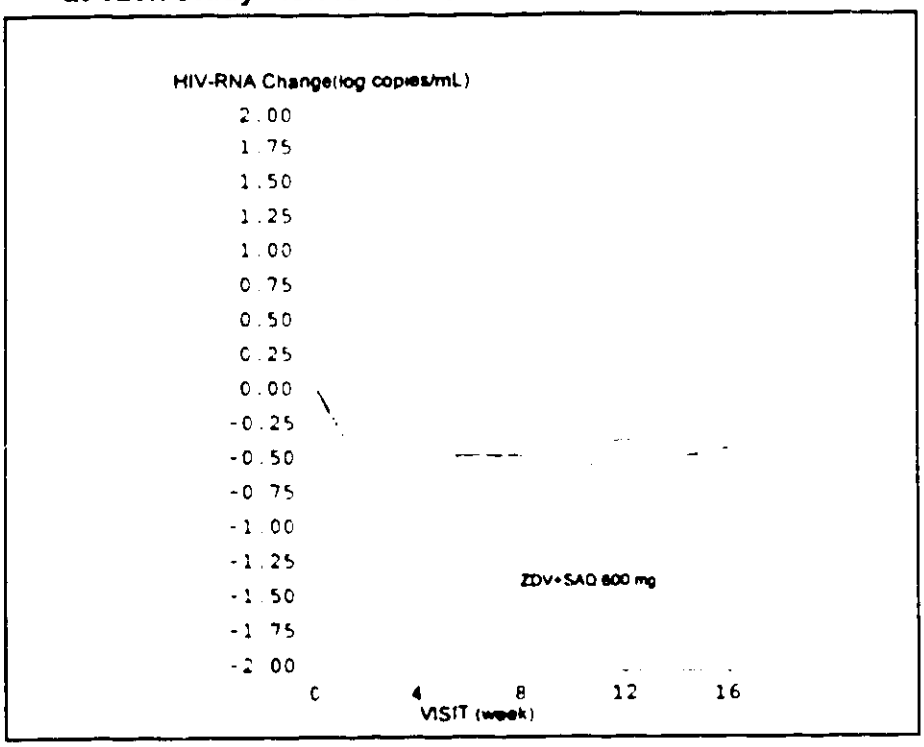
Table 50 shows mean/median changes in log transformed HIV RNA at each study visit for each of the five treatment groups. The primary surrogate analysis period was 16 weeks. Patients randomized to the ZDV+SAQ 600 mg combination achieved the greatest RNA reductions. Combinations of ZDV and SAQ with SAQ doses lower than 600 mg tid produced similar RNA changes to that of ZDV alone. ZDV and SAQ as monotherapy showed similar RNA reductions at week 2, however RNA changes returned toward baseline more quickly among those receiving SAQ. Figure 5 plots mean changes in RNA from baseline at each visit for each of the five treatment groups over 16 weeks of follow-up

Table 50. V-13330 (Italy): FDA ITT population. Mean/median change in HIV-RNA PCR (log copies/mL).

VISIT/WEEK	2	4	8	16	20	28	40	48
SAQ 600mg								
N	19	18	19	18	4	11	9	3
Mean	-0.6	-0.4	-0.3	-0.2	-0.7	-0.4	-0.3	0.0
Median	-0.6	-0.3	-0.2	-0.1	-0.7	-0.4	-0.3	-0.1
Placebo-ZDV								
N	17	16	17	16	04	10	9	2
Mean	-0.6	-0.5	-0.5	-0.6	-0.4	-0.5	-0.2	-0.1
Median	-0.6	-0.6	-0.5	-0.5	-0.5	-0.4	-0.1	-0.1
SAQ 075mg-ZDV								
N	18	15	17	13	6	9	5	2
Mean	-0.7	-0.5	-0.5	-0.5	-0.6	-0.4	-0.5	-0.2
Median	-0.7	-0.5	-0.4	-0.4	-0.7	-0.4	-0.5	-0.2
SAQ 200mg-ZDV								
N	17	17	18	14	7	12	6	2
Mean	-0.8	-0.6	-0.5	-0.4	-0.6	-0.5	-0.4	-0.8
Median	-0.8	-0.5	-0.5	-0.5	-0.7	-0.5	-0.6	-0.8
SAQ 600mg-ZDV								
N	19	19	17	18	7	12	10	2
Mean	-1.6	-1.6	-1.3	-1.0	-1.6	-1.1	-0.7	-1.3
Median	-1.6	-1.6	-1.1	-0.7	-1.6	-0.8	-0.6	-1.3

Source generated from CANDA

Figure 5. V-13330 (Italy): Plot of log transformed RNA changes from baseline at each study visit for five treatment arms.



Source Kazem Kazempour, SAS applications

Table 51 shows the mean change in log transformed RNA from baseline averaged over a 16 week time period The ZDV+SAQ 600 mg combination produced the greatest reduction Overall, there was a statistically significant difference between treatment arms with respect to change in HIV-RNA from baseline In contrast to that seen for CD4 change, there were no differences between study centers with respect to RNA change (See table 52)

Table 51. V-13330: FDA ITT Population. Mean change in HIV-RNA PCR (log copies/mL) over a 16 Week Period (DAVG-16).

TREATMENT	N	MEAN	95% C.I.*
SAQ	19	-0.3	-0.7, 0
ZDV	17	-0.5	-0.9, -0.1
SAQ 75 mg + ZDV	18	-0.5	-0.8, -0.1
SAQ 200 mg + ZDV	18	-0.5	-0.9, -0.1
SAQ 600 mg + ZDV	20	-1.3	-1.6, -0.9

Source: Kazem Kazempour, SAS applications
Scheffe's confidence intervals

Table 52. Overall comparisons for change in RNA.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	10.46	2.61	13.14	0.0001
Center	4	1.27	0.32	1.60	0.1831

Source Kazem Kazempour, SAS applications

Table 53 shows selected pairwise comparisons groups for changes in RNA from baseline The ZDV+SAQ 600 mg tid combination was statistically superior to all other treatment groups for RNA reduction. It is not known if these reductions in RNA are clinically significant. There was no statistical difference between SAQ 600 mg tid alone and ZDV alone. Although SAQ appeared to produce similar or slightly better CD4 increases than ZDV, ZDV appeared to have slightly more activity than SAQ monotherapy in the suppression of RNA-PCR

Table 53. Selected Pairwise Comparisons of treatment groups(Scheffe's test).

TREATMENT	Difference in Means	95% C.I.**
SAQ 600 mg + ZDV vs. ZDV	-0.8	-1.2, -0.3
SAQ 600 mg + ZDV vs. SAQ	-0.9	-1.4, -0.5
SAQ 600 mg + ZDV vs. SAQ 075 mg + ZDV	-0.8	-1.3, -0.3
SAQ 600 mg + ZDV vs. SAQ 200 mg + ZDV	-0.8	-1.2, -0.3
SAQ 600 mg vs. ZDV	0.1	-0.3, 0.6

**Scheffe's confidence intervals

8.2.2.6.4 Safety comparisons

Table 54 below lists the number of patients per treatment group experiencing adverse events of any intensity and at least remotely related to treatment during the 16 week study period. Event categories are listed only if more than one individual in any one treatment group had the event. In general, there were fewer adverse events on the SAQ monotherapy arm compared to the ZDV monotherapy arm. SAQ did not appear to add to the toxicity of ZDV.

Table 54. V-13330: Number (percentage) of subjects with clinical adverse events at least remotely related to trial treatment.

Listed if experienced by more than one individual in at least one treatment group

EVENT	SAQ N=19	ZDV N=17	ZDV-SAQ 75mg N=18	ZDV-SAQ 200mg N=18	ZDV-SAQ 600mg N=20
ALL BODY SYSTEMS	10 (53)	16 (94)	15 (83)	15 (83)	15 (75)
Nausea	2 (11)	10 (59)	11 (61)	9 (50)	9 (45)
Abdominal Pain	1 (5)	4 (24)	4(22)	4 (22)	3 (15)
Vomiting	1 (5)	2 (12)	3 (17)	4 (22)	2 (10)
Diarrhea	2 (11)	1 (6)	4 (22)	1 (6)	1 (5)
Pyrosis	-	2 (12)	-	-	2 (10)
Asthenia	2 (11)	2 (12)	8 (44)	3 (17)	6 (30)
Fever	1 (5)	2 (12)	2 (11)	4 (22)	2 (10)
Appetite loss	-	-	-	3 (17)	-
Headache	3 (16)	5 (29)	9(50)	2 (11)	2 (10)
Dizziness	2 (11)	-	-	2(11)	-
Folliculitus	-	1(6)	-	1(5)	2(10)
Pruritus	1 (5)	-	2(11)	1 (6)	-
Erythema	-	1 (6)	2 (11)	1 (6)	-
Somnolence	1(5)	1 (6)	1(6)	1(6)	2(10)
Insomnia	-	1 (6)	1 (6)	2 (11)	-

Source: CANDA FSR for V-13330. W144918 Table 33 pages 84-88.

Adverse events occurring in greater than 10% (at least two individuals) of subjects taking SAQ monotherapy included nausea, diarrhea, asthenia, headache and dizziness. Nausea and abdominal complaints were reported in more individuals on the ZDV-containing arms. There was no SAQ dose

response relationship for the occurrence of adverse events. Of the events listed in Table 54, eleven were considered to be severe in intensity. Six of the eleven were classified as unrelated, 2 remotely related, 2 possibly related (nausea) and one probably related (nausea and vomiting). The events that were possibly or probably related occurred on ZDV containing treatment regimens.

The most commonly reported adverse events (occurring in at least 10% of subjects) among subjects participating in the double-blind extension phase (day 113 to end of double-blind period) were fever (11%) and asthenia (11%).

Deaths

There were no deaths during the 16 week study period or during the double-blind extension phase of the study

Serious Adverse Events

The final study report states that 9 subjects had serious adverse events during the 16 week treatment phase Eight were classified as unrelated to study treatment, one was remotely related (fever). There was one pregnancy that occurred after 2 weeks of treatment with ZDV + SAQ 75 mg that the sponsor classified as a serious adverse event In the double blind extension phase, 11 patients were reported to have serious adverse events. Four of these events occurred in subjects receiving ZDV monotherapy.

Adverse events leading to premature withdrawal

Table 55 lists the patients who withdrew from study secondary to adverse events. Four patients experienced adverse events which led to premature withdrawal during the 16 week phase and 5 during the double-blind extension phase. In addition two subjects were removed for pregnancy, one during the first 16 weeks and one during the extension phase Four patients discontinued treatment during the 16 week phase and four during the extension phase secondary to laboratory abnormalities (see below)

Table 55. V-13330: Adverse Events leading to Premature withdrawal during entire study period.

Pt. ID	Treatment	Event(s) leading to withdrawal
114	ZDV-SAQ 75 mg	nausea and vomiting
128	ZDV-SAQ 75 mg	erythematous rash
301	ZDV-SAQ 600 mg	CMV gastritis
326	SAQ 600 mg	Lymph node TB
427	SAQ 600 mg	fever, appetite loss, asthenia, chest pain and dyspnea
321	ZDV 200 mg	fever
429	ZDV-SAQ 75 mg	epigastric abdominal pain
401	ZDV-SAQ 200 mg	fever, cough, dizziness, nausea, asthenia, splenomegaly
428	ZDV-SAQ 200 mg	leishmaniasis

Source: CANDA FSR for V-13330, W144'918 (16 week period) pg. 91 and W144'933 (double-blind extension) pg. 65.

Laboratory Abnormalities

Table 56 lists the number of patients who experienced marked laboratory abnormalities during the study. The definition of "marked laboratory abnormality" is the occurrence of a grade 3 or 4 laboratory in a subject whose baseline value was no greater than grade 1. Laboratory abnormalities commonly ascribed to ZDV use, such as low hemoglobin, elevated MCV, low total WBC, neutropenia were reported in the ZDV containing arms but not the SAQ monotherapy arm. Elevated transaminases were reported in all arms except for the combo-75 arm. Six cases of elevated transaminases were reported in subjects on arms containing 600 mg of SAQ.

Table 56. V-13330: Marked Laboratory Abnormalities.

Marked Laboratory Abnormality	ZDV	Combo 75mg	Combo 200mg	Combo 600mg	SAQ 600mg
Hemoglobin	2	2	2	-	-
leucocytes			2		
MCV	7	5	9	5	-
neutrophils	1	2	1	-	-
platelets	1	-	-	-	-
reticulocytes (high)	-	-	-	-	1
alkaline phosphatase	-	-	1	-	-
low calcium	3	-	3	2	2
high phosphate	-	-	-	2	3
low potassium	-	-	-	1	2
low phosphate	2	1	1	3	2
SGOT	1	-	1	2	2
SGPT	1	-	2	3	3
CPK	-	-	-	1	1

Source: generated from CANDA

Comments:
There was no obvious SAQ dose-related effect on any laboratory parameters, although there was a slightly higher number of grade 3 and 4 elevations in SGOT and SGPT in treatment arms containing 600 mg of SAQ in the double-blind extension phase. However the number of patients overall having transaminase elevations was small.

Laboratory abnormalities leading to premature withdrawal from the study
Four subjects prematurely discontinued drug during the 16 week phase secondary to laboratory abnormalities. Three of these four (on ZDV-containing arms) discontinued treatment secondary to anemia. The remaining subject (on SAQ 600 mg) discontinued therapy due to elevated transaminases. This subject was a hemophiliac who was hepatitis B surface antigen positive.

Four subjects withdrew from the double blind extension phase because of a laboratory abnormality. Two patients (on ZDV-containing arms) withdrew for

anemia and neutropenia. One patient (on SAQ 600 mg) with abnormally low platelets at baseline withdrew secondary to persistent thrombocytopenia. The remaining subject (on SAQ 600 mg) withdrew secondary to a grade 4 elevation of transaminases.

8.2.2.3 Reviewer's Conclusions

This study was designed as a phase 1/2 dose ranging-study comparing both SAQ as monotherapy and ZDV plus SAQ (at several doses) to a standard ZDV monotherapy control in antiretroviral-naive individuals. It was not designed with power to detect statistically significant differences between treatment arms. Roche intended to use this study to explore the activity of several doses of SAQ in combination with ZDV and to serve as a guide in designing their phase 2 and 3 studies.

Twelve of the 92 patients (who received drug) discontinued drug or withdrew from study during the 16 week primary surrogate analysis period. However, at least partial data for 92 patients were available for the CD4 and HIV-RNA analyses. One center (Bari) had poor quality control in the performance of CD4 measurements. Values from samples drawn over one two week period were suspect. Roche chose to exclude the 21 patients from this center for their CD4 analysis. FDA includes these for reasons stated above in section xx. The exclusion or inclusion of these individuals does not change the overall conclusions regarding CD4 changes

Activity

Both the increases in CD4 and reductions for HIV-RNA in these treatment-naive patients were greater than that observed in ZDV-experienced patients receiving the same treatments in other trials For both primary activity markers the ZDV+SAQ 600 mg tid combination showed the most activity. For the analysis evaluating the mean change in CD4 from baseline, the ZDV+SAQ 600 mg tid treatment produced the greatest mean increase The CD4 increase from baseline averaged over 16 weeks for this treatment was approximately 80 cells/mm^3. SAQ alone and ZDV+SAQ 200 mg tid produced slightly greater mean increases than ZDV alone and the other combinations SAQ combinations. None of the differences in CD4 changes were statistically significant.

For the analysis evaluating mean change in HIV-RNA from baseline, the ZDV+SAQ 600 mg tid treatment produced the greatest mean reduction compared to all the other treatments The mean RNA reduction from baseline averaged over 16 weeks for ZDV+SAQ 600 mg tid was 1.2 log (copies/mL). These comparisons achieved statistical significance. For changes in HIV-RNA, ZDV with doses of SAQ less than 600 mg tid produced no greater activity than ZDV alone ZDV alone produced slightly greater mean reductions in RNA than SAQ, however this comparison was not statistically significant.

Therefore, evaluation of changes in two surrogate markers indicated that ZDV+SAQ 600 mg produced superior activity compared to ZDV alone, the "standard" treatment for naive individuals at that time. In addition, lower doses of SAQ in combination with ZDV showed no greater activity than ZDV alone for HIV-RNA. It is not clear how the activity of SAQ monotherapy compares to ZDV monotherapy. CD4 changes were slightly greater for SAQ compared to ZDV, but RNA changes were slightly less.

Safety

A smaller percentage of patients randomized to SAQ had adverse events compared to those randomized to ZDV alone or ZDV combinations. SAQ did not increase the frequency, pattern or severity of events associated with ZDV. Adverse events occurring in greater than 10% (at least two individuals) of subjects taking SAQ monotherapy included nausea, diarrhea, asthenia, headache and dizziness. There was no SAQ dose response relationship for the occurrence of adverse events.

There were a few cases of marked transaminase elevations on SAQ containing arms. Two patients withdrew from study for this reason. One of these individuals was hepatitis B surface antigen positive. There was no clear dose response for elevation of transaminases. The number of patients experiencing this abnormality were small, and some had underlying diseases that could contribute to transaminase elevations. One should refer to larger data bases to evaluate the comparative safety of SAQ with regard to transaminase elevations.

8.2 Indication #2 Monotherapy

8.2.1 Protocol O-13328 (United Kingdom)

"A randomized phase i-II, double blinded study, to assess the anti-viral activity, tolerability and pharmacokinetics of oral Ro 31-8959 (HIV proteinase inhibitor) in previously untreated HIV-infected individuals, either minimally symptomatic or with asymptomatic disease and at risk of disease progression, treated at doses of 25, 75, 200 and 600 mg thrice daily."

8.2.1.1 Protocol

8.2.1.1.1 Objectives

The primary objectives of this study were to investigate the SAQ dose response relationship as measured by:

1. antiviral and immunologic effects at four different dose levels
2. tolerability at four different dose levels, documenting clinical and laboratory adverse events

Secondary objectives included assessing the pharmacokinetic profile of SAQ and investigating the feasibility and usefulness of quantitative viremia titration and quantitative RNA/DNA PCR as new laboratory techniques for assessing the antiviral effects of SAQ

8.2.1.1.2 Design

The design is a single center, 16 week, parallel, randomized, double-blind, phase 1/2 study with optional double-blind extension periods. The protocol planned for the enrollment of 40 evaluable patients (up to 60 patients, expecting drop-outs) Patients were randomized to receive one of the four following doses of SAQ

- 25 mg tid
- 75 mg tid
- 200 mg tid
- 600 mg tid

Samples for T-cell subsets and p24 antigen were obtained on 2 consecutive occasions at least 72 hours apart within the 30 day screening period, the last sample obtained within 14 days of the patient's first dose. Measurements of antiviral activity were performed on weeks 1, 2, 3, 4, 8, 12, and 16.

Randomization was stratified by clinical status asymptomatic vs. symptomatic

Subjects were allowed to continue in the double-blind extension phase if they showed evidence of stabilization or improvement in markers of disease activity. One month extensions of therapy were permitted until the database was closed and the blind was broken.

8.2.1.1.3 Population
Eligible subjects were either asymptomatic or minimally symptomatic males with CD4 counts ≤ 500 cells/mm^3. Patients were excluded for any current or previous AIDS-defining condition or for previous antiretroviral treatment. ZDV-experienced subjects would only be enrolled if enrollment into the protocol was found to be slow or incomplete. Of note, this protocol excluded hemophiliacs

Comment:
This was the first SAQ protocol. It began in the U.K. before Roche had submitted their U.S. IND. This protocol had some unreasonable exclusion criteria. Excluding women and hemophiliacs would currently not be consistent with FDA recommendations.

8.2.1.1.4 Endpoints
The primary activity endpoints specified in the protocol were:

Immunologic
CD4 lymphocyte counts (absolute and percent), CD8 and CD4/CD8 ratio.

Virologic
p24 antigen, serum β-2 microglobulin and neopterin.

Secondary endpoints were changes in quantitative PCR using plasma (RNA) and mononuclear cells (DNA) and quantitative plasma and mononuclear cell viremia

Safety endpoints included clinical adverse events and laboratory abnormalities. Clinical adverse events were graded on a three point scale, mild, moderate and severe, according to the WHO grading system. Laboratory abnormalities were also classified according to a modified WHO grading system from grade I to Grade IV. Grade III toxicity (for nonhemologic parameters) or grade IV (for hematologic parameters) were considered severe, necessitating dose interruption or permanent discontinuation of study drug

8.2.1.3.5 Statistical considerations

The protocol specified that antiviral activity would be analyzed according to the following defined responses (nominal dependent variables):

- For p24: 70% reduction (confirmed by a second evaluation 4 weeks later).
- For CD4: an increase of ≥ 30 cells/mm^3 (confirmed by a second evaluation 4 weeks later).
- For viremia: a five-fold reduction in either quantitative viremia or quantitative RNA/DNA PCR titers confirmed by a second evaluation 4 weeks later.

Comment:

Our division has discouraged the use of arbitrarily defined response rates as criteria for analyzing markers of antiviral activity. Instead, we prefer comparisons of mean changes from baseline over time. One such analysis is the DAVG, a weighted average of changes from baseline over time. Although this protocol was originally designed to be a phase 1/2 dose-ranging study, Roche has included a DAVG analysis of CD4 counts in their final study report.

8.2.1.4 Results

The 16 week analysis period of this trial was conducted from Aug., 1991 through Nov., 1992. The double blind extension phase was conducted from Nov., 1992 through Feb., 1993

8.2.1.4.1 Patient Disposition

Forty nine patients were enrolled and randomized, 41 completed 16 weeks of treatment. Reasons for withdrawal from study drug prior to week 16 are described in the Table 57 below. Thirty-three of the 40 subjects who entered the double blind extension phase completed this portion of the protocol. Data from the double-blind extension phase of the study includes subjects who received study treatment ranging from 16-75 weeks.

Table 57. O-13328: Disposition (first 16 weeks) of Patients Randomized.

Dose group	Randomized	Completed	Withdrawals (first 16 weeks)
25 mg tid	12	8	1 overdose 1 AE/intercurrent illness 1 administrative (moved away) 1 lost to follow-up
75 mg tid	12	11	1 insufficient therapeutic response
200 mg tid	13	12	1 AE/intercurrent illness
600 mg tid	12	10	1 overdose 1 administrative (moved away)
Total	49	41	–

Source: CANDA FSR for O-13328, W-144'917, p.25.

8.2.1.4.2 Subject Demographics and Baseline Comparability

All subjects were male, 46/49 were Caucasian. The four treatment groups were comparable with respect to age, race, and weight. The table below shows the median and mean baseline CD4 counts for each dose group. Median/mean baseline CD4 counts were higher in the 25 mg and 600 mg groups compared to the other two treatment arms.

Table 58. O-13328: Summary of Baseline CD4 Counts (cells/mm3), ITT Population.

Treatment	SAQ 025mg	SAQ 075mg	SAQ 200mg	SAQ 600mg
Baseline*				
N	12	12	13	12
Mean	364	230	276	299
Median	375	219	291	340

*Baseline is Median of Screening visits and day 1

Source: CANDA FSR for O-13328, W144'917, page 36, Table 4A.

8.2.1.4.3 Pharmacokinetics

According to Roche's summary, few patients who received the lowest dosage, 25 mg tid, had day 1 SAQ plasma concentrations above the minimum quantification limit of 0 5 ng/mL Some subjects in the 75 mg group and one patient in the 200 mg tid dosing group also had non detectable SAQ concentrations on day 1 Roche states that, "By Day 28, there had been a general increase in plasma concentrations at all dosages although the 25 mg tid dosage still only gave detectable concentrations sporadically."

Comment:

Since individuals in the 25 mg group rarely had detectable levels of SAQ,

this group could be considered as a "placebo control" in some exploratory analyses.

8.2.1.4.4 Efficacy endpoint outcomes

Immunologic, CD4

Table 59 shows mean/median changes in CD4 from baseline over 16 weeks of patient visits. Roche defined baseline CD4 as the median of the two screening assessments plus Day 1. The number of patients per treatment arm was small and the standard deviation of mean CD4 values are large. The only dose group with consistent increases above baseline over the 16 week surrogate analysis period is the 600 mg tid dose. No strong dose response is apparent, but the highest dose appears to produce increases above baseline.

Table 59. O-13328: ITT Population. Mean/median changes in CD4 cells from baseline.

Visit	1	2	3	4	8	12	16
SAQ 25mg							
N	10	11	9	11	10	9	8
Mean	20	-3	27	-5	-48	-46	-6
Std Dev	120	98	165	112	72	90	89
Median	-28	14	-22	-26	-34	-52	-16
SAQ 75mg							
N	10	10	9	12	12	10	11
Mean	-14	-3	11	-18	-33	-44	-33
Std Dev	27	50	94	54	38	47	22
Median	-17	-9	-30	-17	-31	-34	-32
SAQ 200mg							
N	13	12	11	10	12	11	10
Mean	-7	-1	33	17	-21	-17	-23
Std Dev	80	39	74	46	56	92	48
Median	3	7	30	6	-24	-26	-24
SAQ 600mg							
N	9	8	9	10	11	11	10
Mean	36	40	25	88	49	16	5
Std Dev	76	28	79	105	79	75	87
Median	15	39	4	52	33	6	-10

generated from SAS applications of CANDA
Baseline is Median of Screening Visits and Day 1

Table 60 shows the mean change from baseline in CD4 cells averaged over

16 weeks as calculated by the DAVG-16 metric. Confidence intervals are included. For all treatment arms, 95% confidence intervals included zero change from baseline.

Table 60. O-13328: FDA ITT analysis. Mean change in CD4 cells averaged over 16 weeks (DAVG-16) by treatment.

TREATMENT	N	MEAN	95% C.I.*
SAQ 25 mg tid	12	3	-42, 48
SAQ 75 mg tid	12	-20	-65, 25
SAQ 200 mg tid	13	-13	-56, 30
SAQ 600mg tid	12	33	-12, 78

Source: Kazem Kazempour, SAS applicabons
Scheffe's confidence intervals

Overall, there was a statistical trend (see Table 61) toward a difference in the 4 dose groups with respect to changes in CD4 from baseline. Since the difference did not achieve statistical significance for the overall comparison, pairwise comparisons are not included However, there is evidence that the 600 mg tid dose was associated with increases in CD4 above baseline.

Table 61. O-13328, FDA ITT analysis. Overall comparisons.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	3	19785 46	6595 15	2 80	0.0508

Source Kazem Kazempour, SAS applicabons

Virologic

All patients were p24 antigen negative Due to the early stage of disease of most patients in this trial few had measurable plasma viremia.

Table 62 shows the mean/median baseline RNA in absolute copies/mL and in $\log_{10}$ transformed copies It also show minimum and maximum baseline values. All treatment groups had relatively similar mean baseline $\log_{10}$ RNA levels ranging from 4 62 to 4 86 except for the SAQ 75 mg tid group, which had a mean baseline log RNA of 5 32 It is uncertain whether patients with higher or lower baseline RNA are more likely to have greater reductions in RNA in response to antiretroviral treatment The sensitivity of the RNA assay (200 copies/mL) is such that those with the lowest baseline RNA values in each of the for treatment groups (except for the 600 mg tid group) would not be able to demonstrate a full log drop in RNA

Table 62. Summary of Baseline RNA PCR (copies/mL) - Intent to Treat Population

Treatment	SAQ 25mg	SAQ 75mg	SAQ 200mg	SAQ 600mg
Baseline				
N	12	12	13	12
Mean	72,248	208,972	42,079	58,395
Mean $\log_{10}$	4.86	5.32	4.62	4.76
Median	31,535	166,560	16,830	59,555
Median	4.50	5.22	4.23	4.77
Minimum	840	870	450	3320
Maximum	214,960	712,760	215,580	118,160

Source: CANDA FSR for 0-13328, W144'932, page 43, Table 17.

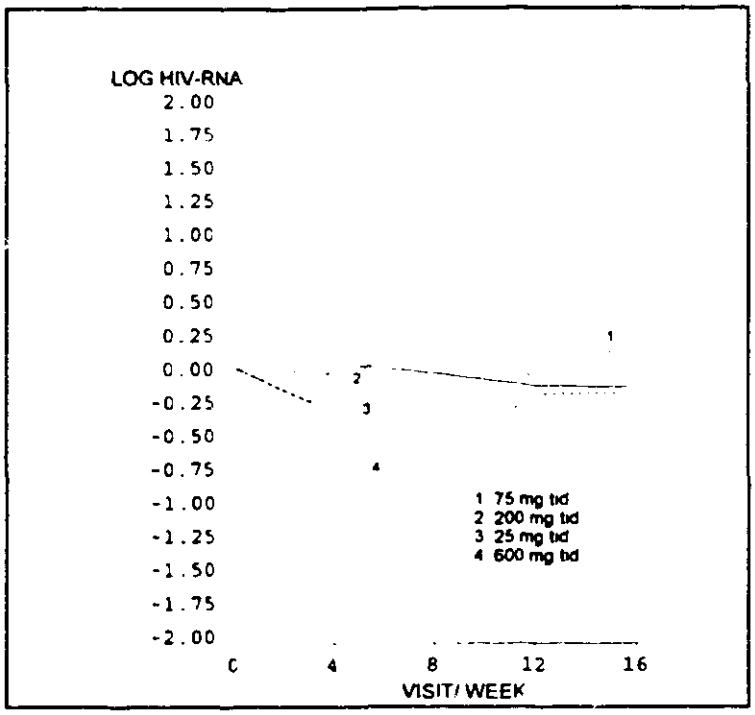
Table 63 shows the mean and median changes in HIV RNA (log transformed copies/mL). Analogous to the changes in CD4 count, the 600 mg tid dose appeared to be active, in this case demonstrating RNA reductions below baseline. Curiously patients receiving the lowest dose of SAQ (where SAQ concentrations were barely detectable) also had slight RNA reductions. This cannot be fully explained based on differences in baseline RNA. The mean baseline RNA value for the 25 mg tid group was in the same range as that for the 200 mg tid and 600 mg tid groups. The numbers of patients in each treatment group were small, so results should be interpreted with caution. Figure 6 plots changes in HIV RNA from baseline over 16 weeks.

Table 63. O-13328: ITT population, mean/median change in HIV-RNA from baseline.

VISIT	WEEK 4	WEEK 8	WEEK 12	WEEK 16
SAQ 025mg				
N	11	9	8	9
Mean	-0.3	-0.2	-0.3	-0.2
Std Dev	0.5	0.3	0.2	0.4
Median	-0.1	-0.2	-0.3	-0.3
SAQ 075mg				
N	11	12	12	11
Mean	0.0	0.1	-0.1	0.2
Std Dev	0.4	0.5	0.7	0.4
Median	0.0	0.1	0.1	0.3
SAQ 200mg				
N	12	12	10	12
Mean	0.0	0.0	-0.1	-0.1
Std Dev	0.4	0.2	0.3	0.5
Median	0.0	0.0	-0.1	-0.1
SAQ 600mg				
N	10	11	10	9
Mean	-0.6	-0.7	-0.2	-0.2
Std Dev	0.8	0.7	0.7	0.7
Median	-0.7	-0.5	-0.3	-0.2

Source generated from CANDA
Baseline is Median of Screening Visits and Day 1

Figure 6. Mean changes in log transformed HIV-RNA over 16 weeks



Source Kazem Kazempour, SAS applications

Table 64 lists mean change in log transformed HIV-RNA averaged over 16 weeks (DAVG-16) with associated 95% confidence intervals. The mean and 95% confidence intervals were below baseline only for the 600 mg tid dose. There was no clear dose response

Table 64. O-13328: FDA ITT analysis, mean change in log transformed HIV-RNA averaged over 16 week (DAVG-16) by treatment.

TREATMENT	N	MEAN	95% C.I.*
SAQ 25 mg tid	12	-0.2	-0.5, 0.2
SAQ 75 mg tid	12	0	-0.3, 0.4
SAQ 200 mg tid	13	0	-0.4, 0.3
SAQ 600mg tid	12	-0.4	-0.7, -0.1

Source: Kazem Kazempour, SAS applications
Scheffe's confidence intervals

Overall, there were no statistically significant differences among dose groups with respect to change in HIV-RNA (see Table 65).

Table 65. O-13328, FDA analysis. Overall comparisons

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	3	3.22	1.07	1.63	0.1958

Source: Kazem Kazempour, SAS applications.

8.1.1.4.3 Safety comparisons

For the 16 week safety analyses, the time window for collection of safety data was up to 4 days following the final dose of trial treatment. The time window for reporting of deaths was up to 28 days following the final dose of trial treatment.

Table 66 shows the duration of treatment exposure through the double blind extension phase. Before the end of the double-blind extension phase, 3 patients received more than 72 weeks of treatment.

Table 66. O-13328: Extent of Exposure to Trial Treatment, ITT population.

SAQ dose	25 mg	75 mg	200 mg	600 mg
Duration (weeks)				
0 - 4	12	12	13	12
>4 - 8	11	12	13	12
>8 - 12	9	12	12	12
>12 - 16	8	11	12	10
>16 - 20	7	11	12	10
>20 - 24	7	10	11	10
>24 - 28	7	10	11	10
>28 - 32	6	10	10	10
>32 - 36	5	9	10	8
>36 - 40	5	8	9	6
>40 - 44	4	6	8	6
>44 - 48	4	6	8	5
>48 - 52	3	5	8	5
>52 - 56	3	5	6	5
>56 - 60	2	4	4	4
>60 - 64	2	3	4	4
>64 - 68	2	3	3	4
>68 - 72	1	1	3	3
>72 - 76	0	0	2	1
>76 - 80	0	0	0	0

Source: CANDA FSR for o_133328, W144932, pg 68

Table 67 shows the number of subjects in the 16 week study portion who experienced at least one adverse event (at least remotely related) and also lists specific types of adverse events if reported in at least two subjects receiving any dose of SAQ. For the 16 week study and the double blind extension phase, there was no dose response relationship for adverse events at least remotely related to trial treatment

Table 67. Number (%) of Patients with Adverse Events (at least remotely related) during 16 week trial period.
Specific types of events are listed if reported by more than one subject

Event	25 mg N=12	75 mg N=12	200 mg N=13	600 mg N=12
Patients with at least one AE, at least remotely related	6 (50)	7 (58)	8 (61)	6 (50)
nausea	1 (8)	2(17)	-	-
flatulence	-	1 (8)	1 (8)	2 (17)
dysethesia	1 (8)	-	1(8)	-
headache	-	-	2 (15)	-
rash (pruritic)	-	1 (8)	1 (7)	-

Source: CANDA FSR for O-13328, Table 26, pp 63-65.

Adverse events for the double-blind extension phase were similar to that of the first 16 weeks. There were no additional specific types of adverse events occurring in more than one individual except for diarrhea, which occurred in two patients (25mg and 600 mg group) in the double blind extension phase.

Deaths
There were no deaths during the 16 week or during the double-blind extension phase.

Serious Adverse Events
Eight subjects experienced adverse events that were considered serious (regulatory definition) by the investigator. Four of these occurred during the first 16 weeks of therapy

- First 16 weeks
  - #101 (600 mg) bleeding from a cutaneous KS lesion.
  - #106 (25 mg) suicide attempt with drug overdose (took 63 capsules).
  - #109 (600 mg) suicide gesture, alcohol use, drug overdose (took 40 capsules)
  - #122 (25 mg) pulmonary infection with hemoptysis.

- Double-blind extension
  - #19 (25 mg) Hospitalization for severe headaches, fever, widespread arthralgia and skin rash. Was able to resume treatment.
  - #17 (75 mg) Hospitalized for chest pain, attributed to anxiety.
  - #12 (200 mg) Diarrhea abdominal pain, nausea, general malaise and intermittent fever. Hospitalized, with worsening of existing symptoms, vomiting, and slight shortness of

breath. Six weeks after withdrawal, the patient developed severe pyomyositis at three sites, due to a Straphylococcus aureus infection.

- #14 (600 mg) fever, rash and malaise, admitted to hospital with fever, and a maculo-papular rash present all over his body and on the soles of his feet.

Adverse events leading to premature withdrawal from the study
Four subjects withdrew from the study prematurely during the first 16 weeks secondary to adverse events. This included two subjects who took overdoses (#106 and #109), subject #122 who withdrew secondary to a pulmonary infection after 51/2 weeks of treatment, and subject #129 who developed a maculopapular rash on day 17 which resolved in 5 days, however the patient withdrew from the study after 6 weeks of treatment.

Two subjects withdrew from the double-blind extension phase secondary to an adverse event; subject #12 (200 mg) developed severe diarrhea and vomiting. Subject #18 (200 mg) was withdrawn at the end of week 20 due to persistent mild peripheral neuropathy in both hands, first reported as dysesthesia in week 12. The investigator reported that there was a possible relationship to treatment.

Laboratory
Roche analyzed the data for "marked laboratory abnormalities" in each treatment group These are summarized in Table 68 Roche defined "marked laboratory abnormalities" in this trial as the occurrence of a grade 3 or 4 laboratory value in a subject who had a normal baseline

Table 68. O-13328: Number of Patients with Marked Laboratory Abnormalities.
From baseline through the double-blind extension phase

Laboratory	SAQ 25 mg	SAQ 75 mg	SAQ 200 mg	SAQ 600 mg
Phosphate (low)	2	1	5	5
Phosphate (high)	2	-	1	-
Potassium (low)	-	-	1	-
Calcium (low)	-	-	1	-
CPK (high)	-	-	1	1
Platelets	-	1	-	-
MCV (high)	-	-	-	1

Source CANDA FSR for O-13328, W-144'917 (16 week), pg 71 and W-144'932 (extension phase) pg. 76.

8.2.1.3 Reviewer's Conclusions
This study was a small dose-ranging study in treatment-naive males. It was one

of the first activity studies of SAQ and was designed as a proof-of-concept study before initiation of phase 2 and 3 studies. Patients who were randomized to the 600 mg tid dose of SAQ, the highest dose studied in the first three phase 1/2 dose ranging studies, had a mean CD4 increase from baseline averaged over 16 weeks of 33 cells/mm^3. The 95% confidence intervals included zero, therefore this increase from baseline did not achieve statistical significance. Comparisons with lower doses also did not achieve statistical significance for CD4 changes. However, the study was small and had a low power to detect differences among treatment arms. There was no apparent dose response; SAQ 600 mg tid was the lowest dose to show some activity over baseline.

Reductions in HIV-RNA were apparent in the SAQ 600 mg tid dosing group. The DAVG-16 was -0.4 log Curiously, patients receiving the lowest dose (25 mg tid) also had some minimal RNA-reduction (DAVG-16=-0.2). Pharmacokinetic data indicated that patients receiving this dose had barely detectable levels. This degree of reduction could be consistent with variability in the test.

The percentages of patients experiencing at least one adverse event in the 600 mg tid group and the 25 mg tid group were both approximately 50%.

8.2.2 Protocol V-13329 (France)

"A randomized, phase I-II, double-blind study, to investigate the anti-viral activity, tolerability and pharmacokinetics of oral Ro 31-8959 (HIV proteinase inhibitor) in previously ZDV-treated HIV-infected individuals, treated at doses of 75, 200 and 600 mg thrice daily."

8.2.2.1 Protocol

8.2.2.1.1 Objectives

Primary

"To investigate the dose response relationship as measured by antiviral activity and tolerability of SAQ in previously ZDV-treated, HIV-infected patients."

Secondary

- To estimate pharmacokinetic parameters after administration of single and multiple doses of SAQ in HIV-infected individuals.
- To investigate the feasibility of using quantitative RNA-PCR to measure antiviral activity of SAQ

8.2.2.1.2 Design

This was a phase 1/2, 16 week, parallel, randomized, double-blind two-center study in HIV-infected individuals previously treated with ZDV. Subjects were randomized to receive one of three doses of SAQ, 75 mg, 200 mg or 600 mg three times daily In the absence of major clinical disease progression, 1 month extensions of therapy were permitted. A total sample size of 45 evaluable patients was planned To allow for drop-outs, it was planned to enter up to 60 eligible patients into the study; each center was to enroll a minimum of 21 evaluable patients

Stratification variables

Patients were stratified by HIV p24 antigenemia (< 50 pg/mL, ≥ 50 pg/mL); at least 50% of the evaluable patients were to enter the study with p24 antigen (ICD) ≥ 50 pg/mL

Blinding

Blinding of the three dosage groups was accomplished by use of matching placebo capsules

Amendments

The only amendment to this protocol involved changing the timing of a blood sample for pharmacokinetic measurements.

8.2.2.1.3 Population, procedures

Inclusion Criteria

The study population was recruited from HIV-infected outpatients attending Hospital Antoine Beclere or Group Hospitalier, Cochin, France. Each of the two centers were to enroll 30 eligible patients. To be eligible subjects were to be HIV infected males or females, age 18 to 65 years old, previously treated with ZDV (subjects may have become intolerant to or progressed while taking ZDV or elected to discontinue ZDV), and CDC group II, III, or IV. Subjects were required to have a CD4 lymphocyte count > 50 and < 250 cells/mm3 (on two separate occasions at least 72 hours apart) and a Karnofsky performance status of ≥ 60 with normal cognitive function. In addition baseline laboratory evaluations were required to be within an acceptable range as specified by the protocol.

Exclusion Criteria (source: CANDA 16 week SR for V-13329, sections 2.4.3, pg. 13)

- neoplasms other than cutaneous Kaposi's sarcoma or basal cell carcinoma
- previous or current history of serious chronic systemic disorder requiring treatment in the preceding 12 months
- intolerable diarrhea of duration greater than 15 days and requiring treatment within 30 days of starting therapy
- pregnancy or lactation
- current history of serious drug abuse (NB: the use of methadone was not an exclusion criterion)
- HIV-encephalopathy (HIV-dementia, AIDS-dementia, sub-acute encephalitis due to HIV, and multi-focal leucoencephalopathy)
- history of psychiatric disorders, including non-reactive depression
- acute serious opportunistic infections requiring immediate treatment and including tuberculosis, CMV, cerebral toxoplasmosis and pneumocystis carinii pneumonia
- CMV or an atypical mycobacterial infection requiring secondary chemoprophylaxis
- abnormal ECG of clinical relevance
- previous or current treatment with any of the following: ddC, ddI, other experimental antiretrovirals, or immunomodulators including the interferons

Concomitant Medications

Medications prohibited were ZDV (must have been discontinued at least 4 weeks prior to first treatment with SAQ), *ddC, ddI or other experimental* antivirals or immunomodulators, ganciclovir, foscamet, drugs known to induce hepatic enzymes (e.g., rifampicin, unless taken regularly for 3 months prior to start of the study and consistently during the study), drugs known to cause systemic toxicity (e.g., myelosuppressive, hepato-, nephro-, or neurotoxic drugs), systemic treatment with corticosteroids within 30 days of starting therapy, treatment with any investigational agent, or antineoplastic agents or radiotherapy (excluding local skin radiotherapy) within 12 weeks of starting therapy. Chemoprophylaxis for *P. carinii* pneumonia, tuberculosis, toxoplasmosis and candidiasis was allowed (i.e., dapsone, pyrimethamine, co-trimoxazole, pentamidine, isoniazid, amphotericin, fluconazole).

8.2.2.1.4 Endpoints

The primary activity parameters specified in the protocol were:

Immunologic
CD4 lymphocyte counts (absolute and percent), CD8 and CD4/CD8 ratio.

Virologic
p24 antigen, serum β-2 microglobulin and plasma and cellular viremia.

Secondary activity parameters were quantitative PCR using plasma (RNA) and mononuclear cells (DNA) and quantitative plasma and mononuclear cell viremia.

For safety parameters, clinical adverse events were graded on a three point scale, mild, moderate and severe, according to the WHO grading system.

Comment:
See comments from previous trials regarding use of RNA PCR and preferred methods for analyzing endpoints.

8.2.2.1.5 Statistical considerations

The definition of the baseline assessment was the median of screenings and Day 1.

As written in the protocol the antiviral activity would be defined according to a response (nominal dependent variable).

- For p24 70% reduction (confirmed by a second evaluation 4 weeks later)
- For CD4 an increase of ≥ 30 cells/mm^3 (confirmed by a second evaluation 4 weeks later)
- For viremia a "meaningful reduction in either quantitative viremia or quantitative RNA/DNA PCR titers confirmed by a second evaluation 4 weeks later. For all analyses involving viraemia data, logarithmic transforms (base 10) of the individual assessments were used

For the 16 week safety analyses, the time window for collection of safety data was up to 4 days following the final dose of trial treatment. The time window for reporting of deaths was up to 28 days following the final dose of trial treatment

8.2.2.2 Results

The initial 16 week trial was conducted between December 1991 and September

1992. The double-blind extension phase was conducted from March, 1992 to January, 1993.

8.2.2.2.1 Subject Disposition

16 week period

Of the 61 patients enrolled and randomized to treatment, 58 completed the study period; three patients withdrew from study drugs prior to completion of 16 weeks. Table 69 shows the number randomized to each group and the reason for withdrawal prior to week 16.

Table 69. V-13329: Disposition of Subjects Randomized

SAQ Dose	Randomized	Completed 16 weeks	Reasons for withdrawal
75 mg tid	20	18	1 intercurrent illness[a] 1 insufficient efficacy
200 mg tid	21	21	1 administrative error[b]
600 mg tid	20	20	0

Source: CANDA FSR for V-13329, W144'916, p26

a #236 developed cerebral toxoplasmosis at day #6

b #308 was withdrawn after 15 weeks instead of 16 due to an administrative error

Double-blind extension phase

The double-blind extension phase of this protocol was conducted from March, 1992 - January, 1993, after which some subjects switched to open-label SAQ. Table 70 shows the extent of exposure of study drug through the double-blind extension phase. For the first 24-28 weeks, there was little difference among treatment groups in the number of patients dropping out, however after this time there was a larger drop-out rate in the 75 mg group compared to the other groups. One patient in each group received more than one year of treatment.

Table 70. V-13329: Extent of Exposure to Trial Drug, ITT population.

Treatment	75mg	200mg	600mg
Duration (weeks)			
0 - 4	20	21	20
>4 - 8	19	21	20
>8 - 12	19	21	20
>12 - 16	18	21	20
>16 - 20	17	20	19
>20 - 24	15	19	18
>24 - 28	14	16	18
>28 - 32	12	15	16
>32 - 36	7	15	15
>36 - 40	7	10	12
>40 - 44	5	8	9
>44 - 48	5	7	7
>48 - 52	4	5	5
>52 - 56	2	3	2
>56 - 60	1	1	1
>60 - 64	0	0	0

Source CANDA FSR for V-13329, W144931, pg. 54, Table 25.

8.2.2.4.2 Subject Comparability and Demographics

The three treatment groups were comparable with regard to sex and age. Age ranged from 19-64 years Sixteen of the 61 (26%) subjects were women. Homosexual contact was the most common means of HIV transmission in all groups and most subjects had a baseline Karnofsky score of 100 All patients had received previous ZDV therapy; the duration of previous ZDV therapy ranged from 11-1575 days and was comparable in the three treatment groups, median duration of prior ZDV treatment was approximately 400 days (416, 395, and 392 days for the 75 mg, 200 mg, and 600 mg groups, respectively)

The mean/median baseline CD4 counts are shown in Table 71.

Table 71. V-13329: Summary of Baseline CD4 Counts (cells/mm3).

Treatment	SAQ 75mg	SAQ 200mg	SAQ 600mg
Baseline			
N	18	21	20
Mean	168	139	132
Median	171	137	150

Source: CANDA FSR for V-13329; W144'931, pg. 37, Table 4
Baseline is Median of Screening visits and day 1

8.2.2.4.2 Efficacy endpoint outcomes

CD4

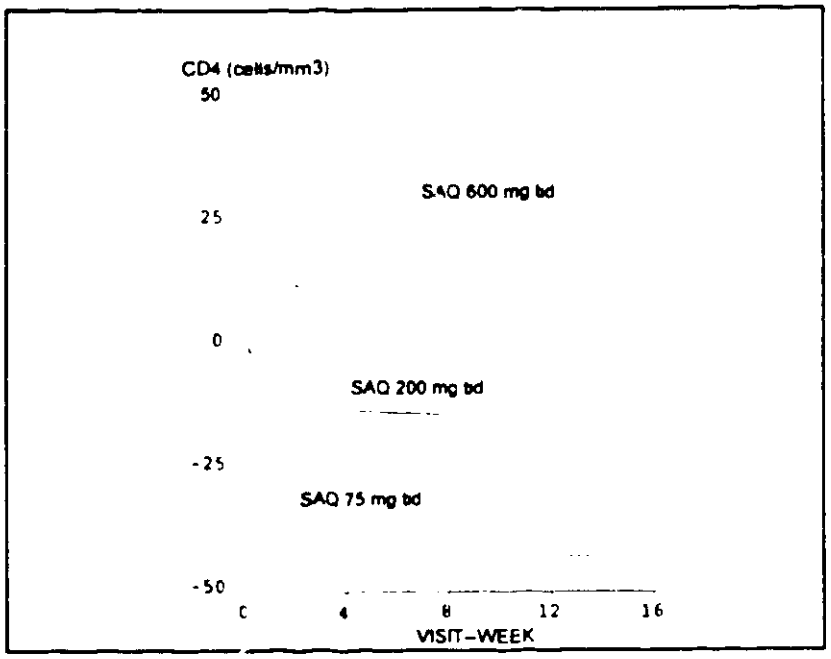
Table 72 shows the mean/median CD4 changes from baseline for each of the first seven visits through week 16 by treatment group. Subjects randomized to the 75 mg tid group showed a (mean/median) decline of CD4 cells through 16 weeks of follow-up. Those randomized to the 200 mg tid dose showed an inital gain in mean/median CD4 cells which declined at week 4 for a net loss at week 16. Those randomized to the 600 mg tid group showed a mean/median gain in CD4 above baseline that peaked at week 3-4 but returned toward baseline. Figure 7 plots mean changes in CD4 cell counts for each of the 7 visits during the 16 weeks of study.

Table 72. V-13329: ITT population, mean/median change in CD4 Counts (cells/mm3) from baseline.

WEEK	1	2	3	4	8	12	16
SAQ 75mg							
N	20	19	19	19	19	19	18
Mean	-8	-13	-12	-25	-28	-42	-37
Median	-8	-12	-13	-26	-43	-29	-29
SAQ 200mg							
N	21	21	21	21	20	21	21
Mean	16	9	7	-17	-17	-26	-18
Median	-17	3	1	-19	-24	-29	-25
SAQ 600 mg							
N	20	20	18	20	20	20	20
Mean	40	50	50	37	22	26	9
Median	32	18	30	29	22	15	6

Source generated from CANDA, baseline is Median of Screening Visits and Day 1

Figure 7. V-13329 (France): mean changes in CD4 cell counts (cells/mm^3) from baseline.



Source Kazem Kazempour, SAS applications

Table 73 shows mean change in CD4 from baseline averaged over 16 weeks by SAQ dose groups Only the 600 mg tid produced a mean increase in CD4 cells from baseline over the 16 weeks of study. The mean change from baseline for this dose was 26 cells/mm^3 The lower 95% confidence interval for this mean change was greater than zero

Table 73. V-13329: FDA ITT analysis, mean change in CD4 from baseline averaged over 16 week by treatment (DAVG-16).

TREATMENT	N	MEAN	95% C.I.*
SAQ 75 mg tid	20	-30	-48, -11
SAQ 200 mg tid	21	-14	-32, 4
SAQ 600mg tid	20	26	7, 45

Source Kazem Kazempour, SAS applicabons. Scheffe's confidence intervals

There was a statistically significant difference among treatment groups with respect to changes in CD4 from baseline There was no significant difference between the two centers with respect to CD4 changes (see Table 74)

Table 74. V-13329: FDA ITT analysis, overall Comparisons.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	2	33656.23	16828.11	19.59	0.0001
Center	1	74.66	74.66	0.09	0.7692

Source: Kazem Kazempour, SAS applications

Table 75 shows pairwise comparisons between dose groups for changes in CD4 counts. SAQ 600 mg tid produced statistically superior CD4 increases compared to the two lower doses.

Table 75. V-13329: FDA ITT analysis, pairwise comparisons of treatment groups.

TREATMENT	Difference in Means	95% C.I.**
SAQ 600 mg vs 200 mg	40	17, 63
SAQ 600 mg vs 75 mg	56	33, 80
SAQ 200 mg vs 75 mg	16	-7, 39

Source: Kazem Kazempour, SAS applications
**Scheffe's confidence intervals

In the protocol, the original definition of a CD4 responder was a patient who maintained an increase of 30 cells/mm^3 for 4 weeks. There was one subject who achieved a 25 25 response in the 200 mg group; this individual did not maintain an increase of 30 cells/mm3 for 4 weeks. In the 600 mg tid group, 7 individuals met the protocol's definition of a responder (increase in CD4 of 30 cells/mm3)

HIV RNA-PCR

Table 76 shows the mean/median baseline RNA levels for each dosing group. RNA-PCR levels were approximately 50,000 copies/mL in the 75 and 200 mg tid groups and 73,000 copies/mL in the 600 mg tid group.

Table 76. V-13329: ITT population, summary of baseline RNA PCR (copies/mL).

Treatment	...ig	SAQ 200mg	SAQ 600mg
Baseline			
N	19	21	20
Mean	200,256	142,504	159,264
Mean $\log_{10}$	5.30	5.15	5.20
Std Deviation	327,853	244,457	234,786
Median	54,454	44,495	72,588

Baseline is Median of Screening visits and day 1
Source: CANDA FSR for V-13329; W144'931, pg. 47.

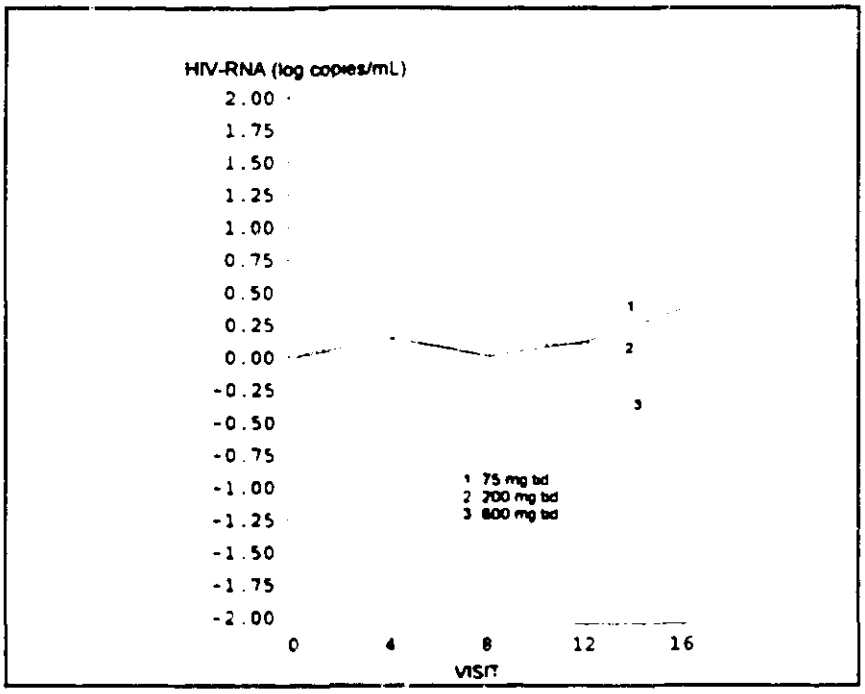
Table 77 shows the mean/median changes from baseline of log transformed HIV-RNA over 16 weeks for each dose group. Figure 8 is a plot of the mean changes over 16 weeks. This plot contains additional data from weeks 1, 2 and 3. A decrease in RNA was seen transiently at week 3 in the SAQ 600 mg tid dose group.

Table 78. V13329: ITT population, mean/median changes from baseline in log HIV-RNA (PCR)

VISIT	WEEK 4	WEEK 8	WEEK 12	WEEK 16
SAQ 075mg				
N	18	17	17	16
Mean	0.2	0 1	0 1	0.2
Std Dev	0.4	0 4	0 8	0.5
Median	0 1	0 2	0 1	0.4
SAQ 200mg				
N	21	19	19	18
Mean	0 1	0 0	0 1	0.4
Std Dev	0 6	0 6	0 6	0.7
Median	0.1	0 1	0 2	0.2
SAQ 600mg				
N	19	19	18	17
Mean	0.2	0 0	-0 2	-0.3
Std Dev	0.7	0 8	0 7	0.6
Median	0.0	0 0	-0 2	-0.2

Source: generated from CANDA

Figure 8. V-13329: mean changes in HIV RNA (log copies/ml) plotted over 16 weeks



Source: Kazem Kazempour, SAS applications

Table 78 shows the mean change in HIV-RNA from baseline averaged over 16 weeks. There were no overall reductions in any group.

Table 78. V-13329: FDA ITT analysis, mean change in HIV-RNA from baseline averaged over 16 week (DAVG-16) by treatment.

TREATMENT	N	MEAN	95% C.I.*
SAQ 75 mg tid	20	0.1	-0.2, 0.4
SAQ 200 mg tid	21	0.1	-0.4, 0.1
SAQ 600mg tid	20	0	-0.6, 0.5

Source: Kazem Kazempour, SAS applications *Scheffe's confidence intervals

Overall, there was no statistically significant difference between dose groups or centers with respect to mean change from baseline in HIV-RNA (see Table 79)

Table 79. V-13329: FDA ITT analysis, overall comparisons.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	2	0.82	0.41	0.53	0.5897
Center	1	4.87	4.87	6.36	0.0145

Source: Kazem Kazempour, SAS applications

8.2.2.4.3 Safety comparisons

Table 80 shows the number of subjects with at least one adverse event at least remotely related to treatment during the 16 week study period and also shows specific types of adverse events (listed if occurring in at least two subjects at any dose). Although the numbers are small, there is no apparent dose-response relationship for adverse events. Diarrhea is the only event which appeared to occur more frequently at higher doses.

Table 80. V-13329: Number of patients (percentage) with clinical adverse events (excluding unrelated) during the 16 week study period.

Specific types of events listed if experienced by two or more subjects.

Event	75 mg (n=20)	200 mg (n=21)	600 mg (n=20)
Total patients with at least one AE	10 (50)	16 (76)	17 (85)
Diarrhea	1 (5)	4 (19)	5 (25)
Abdominal pain	2 (10)	1 (5)	2 (10)
Nausea	2 (10)	-	1 (5)
Fever	1 (5)	4 (19)	2(10)
Dizziness	2(10)	-	-
Enlarged lymph nodes	2 (10)	2 (10)	2(10)
Hypotension, postural	2(10)	1 (5)	-
Flushing	-	1 (5)	3(15)

Source CANDA FSR for V-13329 . W144916 Table 28, pg 69

After 16 weeks there were no additional types of adverse events associated with SAQ

Deaths

There were no deaths during the 16 week study period or within 4 weeks of completion of the study. One patient died during the double-blind extension phase. This was subject #201 who died secondary to a subdural hematoma that developed after a fall. The fall was attributed to his generalized debilitated state.

Serious adverse events

Five patients (shown in Table 81) had serious adverse events during the initial 16 week treatment phase. None of these events were considered to be related to treatment. Only one individual (#236) was removed from treatment during the first 16 weeks for a serious adverse event.

Table 81. V-13329: Serious Adverse Events-first 16 weeks.

Patient Number/dose	Event	Relationship
236 (75 mg tid)	seizures focal brain lesion/ toxoplasmosis	not related
241 (200 mg tid)	diarrhea/microsporidium and cryptosporidium	not related
244 (200 mg tid)	anal fissurectomy	not related
248 (200 mg tid)	cervical conization for dysplasia	not related
304 (600 mg tid)	plastic surgery/scar removal	not related

Source: CANDA FSR for V-13329, W144'916, from text, p74-75.

Thirteen subjects experienced serious adverse events during the double-blind extension phase these were unrelated.

Adverse Events leading to premature withdrawal from the study

From the study initiation through the double-blind extension phase, only one patient withdrew prematurely due to and adverse event. This was patient #236 who developed seizures related to cerebral toxoplasmosis on study day #6. Two additional subjects (#241 and #244) in the 200 mg tid group cited a combination of insufficient therapeutic response, and adverse events/intercurrent illness, as their reasons for withdrawing from the study. Subject #231 dropped out for unknown reasons, during the extension phase.

Laboratory Abnormalities

Table 82 shows marked laboratory abnormalities (defined previously) from baseline to the end of the double blind extension phase.

Table 82. V-13329: Summary of Marked Laboratory Abnormalities through the end of the double-blind extension phase.

LABORATORY	SAQ 75 MG	SAQ 200 MG	SAQ 600 MG
Phosphate (low)	5	4	3
Potassium (low)	2	3	3
Potassium (high)	0	1	0
SGOT	0	1	0
CPK (high)	0	1	1
Neutrophils (low)	1	2	0
Reticulocytes (high)	1	0	0

Source: CANDA FSR for V-13329; W-144'916 (16 week) p.79 and W-144'931 (extension phase) p.66. and verified with SAS applications for CANDA

8.2.2.5 Reviewer's Conclusions of study

This study was one of the initial dose-ranging activity studies. It was designed to explore activity at various SAQ doses. This is the only study that demonstrated, with statistical significance, that SAQ 600 mg tid was superior to lower doses of SAQ monotherapy. for changes in CD4 from baseline. For the 600 mg tid dose, there was a mean CD4 gain over 16 weeks of 26 cells/mm^3. The lower 95% confidence interval this CD4 increase was greater than zero, indicating a statistically significant increase from baseline CD4.

There was no apparent mean RNA reduction for any dose averaged over the 16 week period. There was a transient reduction in the 600 mg tid dose that returned to baseline by week 4

At present it is unknown whether the statistically significant increase in CD4 count from baseline for the 600 mg tid dose is clinically significant. The lack of a reduction in RNA copy number casts doubt regarding the activity of this drug as monotherapy.

There was no clear dose response observed in the dose range used in this study. There was one active dose, 600 mg tid. This study indicated that this dose may be the threshold for activity. Higher doses of this drug may produce greater activity

There were no deaths or serious adverse events that were considered to be related to SAQ over the duration of the surrogate analysis period and double-blind extension. A slightly higher percentage of individuals receiving SAQ 600 mg tid experienced diarrhea. There were no consistent laboratory abnormalities at the 600 mg tid dose compared to lower doses

8.3 Other Studies

8.3.1 EV 14757

8.3.1.1 Protocol Summary

This is an ongoing, single center, open label, dose-ranging study, conducted under an investigator IND (T. Merigan, M.D., U.S.A.) at Stanford University. To be eligible patients were required to have CD4 cell counts between 200 and 500 cells/mm^3 and no more than 12 weeks of prior therapy with ZDV. Those who had previously taken nucleosides other than ZDV were excluded. Patients were sequentially assigned to one of 2 treatment groups:

- SAQ 3600 mg daily dose (600 mg q4h)
- SAQ 7200 mg daily dose (1200 mg q4h).

By June 1, 1995, 20 patients had enrolled (19 male and 1 female) into the SAQ 3600 mg arm and had received treatment ranging from 17-67 weeks (median 35), while 21 patients (all male) had been entered into the SAQ 7200 mg treatment arm and had received treatment ranging from a duration of 2-56 weeks (median 25). Although these patients were allowed up to 12 weeks of previous ZDV treatment. The majority were treatment naive.

8.3.1.2 Preliminary Data

Activity

From preliminary data, Roche reports (in an NDA update) that the 3600mg/day and 7200mg/day doses of SAQ produced greater increases in CD4 cell and greater reductions in HIV-RNA than that of the 600 mg tid dose (1800mg/day). (See Figure 9 under section 9 1 1 for a plot of log transformed SAQ AUCs vs. $\log_{10}$ change in RNA, courtesy of Roche.)

Safety

Table 83 shows the adverse events (all intensities) that occurred among patients enrolled in the two treatment arms These are compared to event rates in a pooled group of treatment naive patients who had received SAQ 1800 mg/day in two European studies (0-13328 and V-13330) Since these were separate studies with different designs (European studies were double-blind and EV14757 was open-label) and since the median duration of treatment in the European studies was greater than that in EV14757, comparisons should be made with caution In summary, events involving the gastrointestinal system, specifically diarrhea, nausea and abdominal pain appeared to occur with greater frequency in both the SAQ 3600 mg/day and 7200 mg/day groups compared to the 1800 mg/day group

Table 83. Number (%) of patients with AEs of all intensities excluding unrelated. Listed if at least 10% in any treatment group Includes data up to June 1, 1995

ADVERSE EVENT *Median Duration of exposure*	SAQ 1800 mg* N=32 *44 wks*	SAQ 3600 mg N=20 *35 wks*	SAQ 7200 mg N=21 *25 wks*
ALL BODY SYSTEMS			
Total Patients with at least one AE	20 (63)	18 (90)	14 (67)
Total number AEs	90	92	62
GASTROINTESTINAL DISORDERS			
diarrhea	5 (16)	9 (45)	10 (48)
abdominal discomfort	2 (6)	7 (35)	4 (19)
nausea	5 (16)	5 (25)	7 (33)
abdominal pain	2(6)	3 (15)	1 (5)
Mucosa Damage	-	-	3 (14)
Tooth disorder	4 (12 5)	1 (5)	-
CENTRAL AND PERIPHERAL NERVOUS SYSTEM			
headache	4 (13)	6 (30)	3 (14)
dizziness	3 (9)	5 (25)	4 (19)
confusion	-	2 (10)	-
numbness extremities	-	2 (10)	-
GENERAL DISORDERS			
asthenia	6 (19)	7 (35)	3 (14)
SKIN AND APPENDAGES			
rash	2 (6)	3 (15)	1 (5)
RESPIRATORY DISORDERS			
Rhinitis	3 (9)	4 (20)	3 (14)
Cough	3 (9)	3 (15)	1 (5)
OTHER			
Micturition disorder	1 (3)	5 (25)	1 (5)
Heart Rate Disorder	2 (6)	2 (10)	1 (5)

ADVERSE EVENT *Median Duration of exposure*	SAQ 1800 mg* N=32 *44 wks*	SAQ 3600 mg N=20 *35 wks*	SAQ 7200 mg N=21 *25 wks*
MUSCULOSKELETAL SYSTEM			
myalgia	1 (3)	2 (10)	1(5)
musculoskeletal pain	-	4(20)	-
arthralgia	-	2 (10)	1 (15)
PSYCHIATRIC DISORDERS			
insomnia	2 (6)	3 (15)	1 (5)

Source: Safety Update pg. 88-92, table 14 and CANDA
*Pooled from studies 13330 and 13328.

Serious Adverse Events

There was only one serious adverse event in EV14757, a suicide attempt at the high dosage group (7200 mg)

Laboratory Abnormalities

Table 84 lists the marked laboratory abnormalities in EV14757 as compared to individuals receiving 1800 mg a day in studies 0-13328 and V-13330. There was no obvious dose related changes in laboratory abnormalities over this dosage range. Roche has defined marked laboratory abnormalities as a shift in 3 toxicity grades, i.e., a grade 3 or 4 abnormality in subjects with a normal baseline or a grade 4 abnormality in a subject with a grade 1 abnormality at baseline.

Table 84. Summary of Marked Laboratory Abnormalities in EV14757 compared to data pooled from European studies.

Marked Laboratory Abnormalities	Percentage of Patients with Marked Laboratory Abnormalities		
	1800 mg n=32	3600 mg n=20	7200 mg n=21
High CPK	1 (3%)	-	1 (5%)
Low Phosphate	6 (19%)	-	-
High ALT	2 (6%)	-	1 (5%)
High AST	2 (6%)	-	1 (5%)
Low Potassium	3 (9%)	-	-
High calcium	1 (3%)	-	-
Low calcium	-	1 (5%)	-

Source Safety update, Appendix 15 and CANDA

8.3.2 SV14604

8.3.2.1 Protocol Summary

SV14604 is an ongoing double-blind, international, randomized, parallel study in HIV-infected patients with pre-entry CD4 counts between 50 and 350 cells/mm^3 and with less than 16 weeks of prior ZDV treatment. There are 4 treatment arms:

- ZDV monotherapy (200 mg),
- ZDV + ddC (0.75 mg),
- ZDV + SAQ (600 mg)
- ZDV + ddC + SAQ (triple combination).

8.3.2.2 Preliminary Safety Data

By the clinical safety data cut-off on June 1, 1995, 1348 patients (82% male, 85% white, with ages ranging from 17 to 73) had entered the trial. The study remains blinded. In response to ACTG 175 results, a recent SV14604 protocol amendment has stopped enrollment into the ZDV arm and switched those patients already enrolled to ZDV monotherapy to triple combination therapy.

There were four deaths, all were judged unrelated to study treatment. Of the 83 serious adverse events 24 were considered to be possibly or probably related to study treatment These are listed in Table 85.

Forty-four (44) patients withdrew from the study secondary to an adverse events: most of these events were nausea and vomiting (19), headache, fatigue or hematologic abnormalities These are adverse events commonly ascribed to ZDV.

Table 85. SV14604, Serious Events possibly or probably related to study treatment.

Possible	Probable
anemia (3 cases)	anemia (6 cases)
convulsions	esophageal ulceration
fever, vomiting, headache	pancreatitis
suicide attempt	penpheral neuropathy
allergic reaction	
pancytopenia	
agranulocytosis*	
neutropenia, phlebitis	
hepatitis	
liver enzyme disorder	

Source Safety Update Appendix 16
*It is unclear from the case report form if this is truly agranulocytosis; drug was discontinued for grade 3 neutropenia; also stated on the case report form is possible ZDV agranulocytosis.

8.3.3 SV14788 (Rollover Protocol)

8.3.3.1 Protocol Summary

This is an open-label, international noncomparative safety study for individuals who have previously taken part in other Roche sponsored studies (except NV14255, which has its own rollover protocol) All patients in this protocol receive SAQ 600 mg tid, with the exception of patients entering from the discontinued SAQ 200 mg + ddC arm from protocol NV14256, who were given the following 4 treatment options no treatment, SAQ 600 mg tid, ddC 0.75 mg, or SAQ 600 mg tid plus ddC

8.3.3.2 Safety data

By the cut-off date of June 1, 1995, a total of 210 patients (mainly white Caucasian 85% and male 88% with ages ranging from 21 to 69) had entered the trial

There were 4 deaths, all considered unrelated to study treatment and related to AIDS A total of 19 serious adverse events were recorded ; 4 were considered at least remotely related to treatment (shown in Table 86). Patient #1351, who developed Stevens Johnson Syndrome, was reported in a 10 safety report (see section 10 1.2)

Table 86. SV 14788: Serious adverse events at least remotely related.

Patient ID	Treatment	Event/outcome	Relationship
14721/115	SAQ (+ZDV)	hospitalized for fever resolved after drug discontinued	remote
14736/1351	SAQ	Stevens-Johnson syndrome resolved	possible
14798/1354	SAQ* +ddC	neutropenia resolved on neupogen	remote
14798/1601	SAQ	decreased visual acuity/macular stippling continued	remote

Source: Safety Update pg 95
*received 200 mg SAQ

Patients Withdrawing for Adverse Events
Three patients withdrew for adverse events or laboratory toxicities. This included patient #1351 with Stevens-Johnson syndrome, one patient (#14751/0007) with vertigo and nausea (remotely related) and one patient (14797/1482) with elevated liver enzymes and thrombocytopenia (unrelated).

8.3.4 NV14802 (NV14255 Rollover Protocol)

8.3.4.1 Protocol Summary
This is an U.S. multicenter, open-label, noncomparative safety study for patients who had previously participated in study NV14255. Patients are receiving SAQ 600 mg + ZDV 200 mg + ddC 0.75 mg. At the clinical cut-off date, a total of 190 patients (176 males and 14 females) were entered into the study.

8.3.4.2 Preliminary Safety Data
There were 2 serious adverse events. The first, considered unrelated to study treatment, was a patient hospitalized for a colonic resection of a stricture secondary to CMV colitis. The second was a patient (15906/102) who required hospitalization for pancreatitis and subsequently died. The pancreatitis was attributed to infection with MAI and was not considered related to study treatment.

Three patients died prior to June 1, 1995. In addition to patient #102 with pancreatitis, a patient (14935/174) died from pneumonia and pulmonary KS (unrelated) and the third (#14998/61) from PCP and Klebsiella bacteremia (unrelated).

In this ACTG 229 roll-over protocol, 7 patients withdrew for adverse events; reasons for withdrawal are known for 4 of the 7. These are: leg cramps (possible), elevation of LFTs (remote), peripheral neuropathy (probable) and glucosuria (possible).

8.3.5 Summary of safety data from ongoing studies and roll-over protocols
Safety data from EV14757 showed that higher doses of SAQ are associated with the same types of adverse events as reported for 600 mg tid dose. However higher doses may be associated with a higher frequency of some gastrointestinal adverse events, such as diarrhea, nausea and abdominal pain.

The safety data that Roche submitted from the ongoing trials, which include roll-over protocols, were serious adverse events, deaths and withdrawals due to adverse events. For study SV14604, these were reported in a blinded-fashion. Most of the events reported from this study and the roll-over protocols are those which have been associated with the use of ZDV and ddC (which were used concomitantly in SV14604 and in some patients in roll-over protocols) or are events, such as liver enzyme abnormalities, that have previously been described in completed SAQ trials The exceptions are two serious adverse events reported in SV14788. The first was a patient with Steven's Johnson Syndrome (possible relationship according to the investigator) and the second was a patient with decreased visual acuity and macular stippling (remote relationship). The case of Steven's Johnson Syndrome is included under adverse events in the product label.

9 Overview of Efficacy-Activity

In the past, accelerated approval for antiretroviral drugs has been based on changes in antiviral activity as measured by changes in CD4 counts. In this case, approval of the applicant's proposed indication for SAQ in combination with nucleoside analogues is based on changes in CD4 cell counts and plasma HIV-RNA. Roche has agreed submit efficacy data with clinical endpoints in an NDA supplement as part of their accelerated approval commitments for SAQ.

9.1 General Issues

The poor bioavailability (4% with food, in healthy volunteers[4]) of the SAQ hard gelatin capsule (HGC) formulation, has been an obstacle in the development of SAQ. The three phase 1/2 European dose-ranging studies did not establish a maximally tolerated dose using this formulation, nor did they demonstrate a clear dose response or plateau of antiviral activity. The 600 mg tid dose was the only dose showing activity in these trials. Consequently, after preliminary data from these trials were available, FDA advised Roche to include exploration of higher exposures of SAQ in their development plan. They acknowledged the importance of this issue but decided to initiate phase 2/3 studies with SAQ 1800 mg/day, HGC. Based on preliminary results from their Italian study, Roche interpreted the activity of 600 mg tid dose of SAQ to be reasonably comparable to that of ZDV in naive patients. Therefore, they decided not to delay the initiation of phase 2 and 3 studies pending the development of a formulation with improved bioavailability. At that time, Roche stated that it was not feasible to either commercialize or study, on a large scale, doses greater than 1800 mg/day (600 mg tid) of the poorly bioavailable HGC formulation for the following reasons

1) manufacturing limitations and
2) patient acceptability of taking a large number of capsules to achieve higher exposures

As the phase 2 and 3 program with the HGC at 1800 mg/day began, Dr. J. Merigan, initiated an open label study, EV14757, using higher doses of SAQ under a separate IND. In this study 41 patients were assigned to either 3600 mg/day or 7200 mg/day of SAQ, HGC. After substantial enrollment of two phase 3 trials, preliminary data from EV14757 became available, suggesting greater antiviral activity in patients achieving higher plasma AUCs of SAQ.

Figure 9 is a plot of average $\log_{10}$ RNA reduction vs 24 hour log transformed AUCs in 15 naive (mostly) individuals receiving SAQ monotherapy in EV14757. Overall, subjects receiving 7200 mg/day achieved higher 24 hour AUCs than those receiving the 3600mg dose. Higher plasma AUC_{24}s were associated with greater reductions

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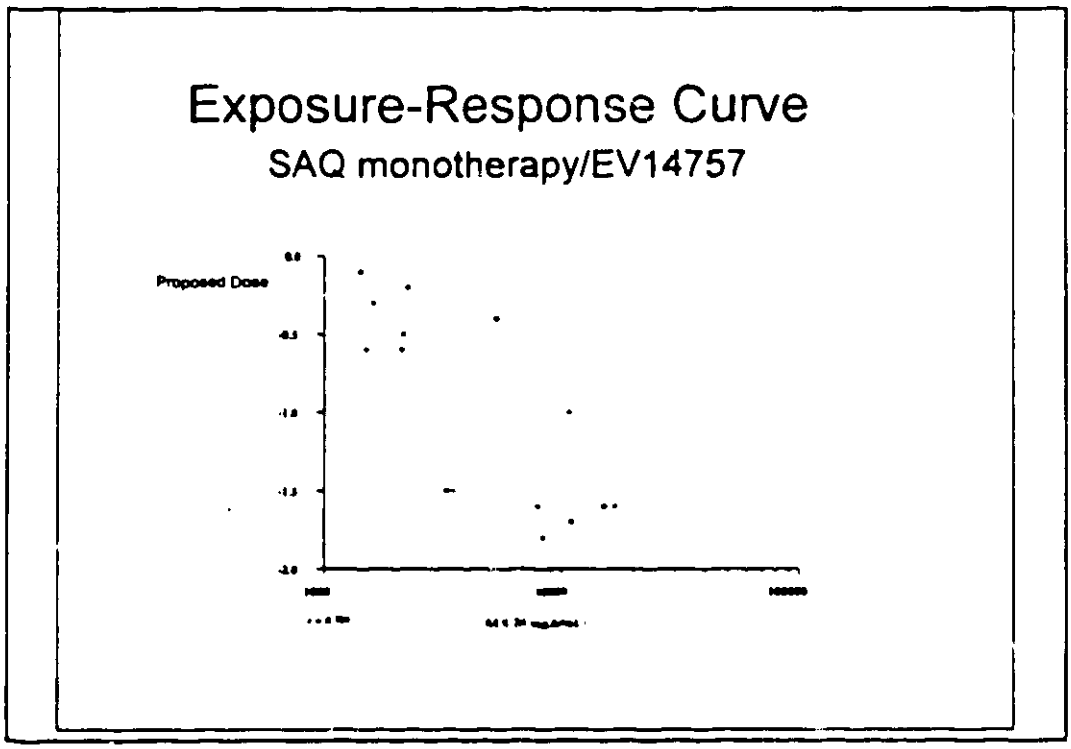
[4] Data is lacking with respect to the bioavailability of SAQ in HIV-infected individuals

in viral RNA. The box in the upper right corner represents an estimated area of exposure-response for the proposed dose of 600 mg tid. According to the phase 1/2 studies, average AUCs at this dose were approximately 2000 ng.hr/ml (range 400 to 2700), average reductions in viral load range from zero to less than 0.5 log RNA copies/mL. This plot gives an indication of the limitations of the current formulation at the proposed dose and also the future possibilities of higher exposures with improved formulations.

Fig. 9. Exposure response curve for SAQ.



Source: Courtesy of Roche, NDA update (10/9/95) Fig. 2, pg. 4. Box estimating area of AUC and RNA changes for proposed dose was added by FDA medical reviewer

9.2 Activity, Combination Therapy

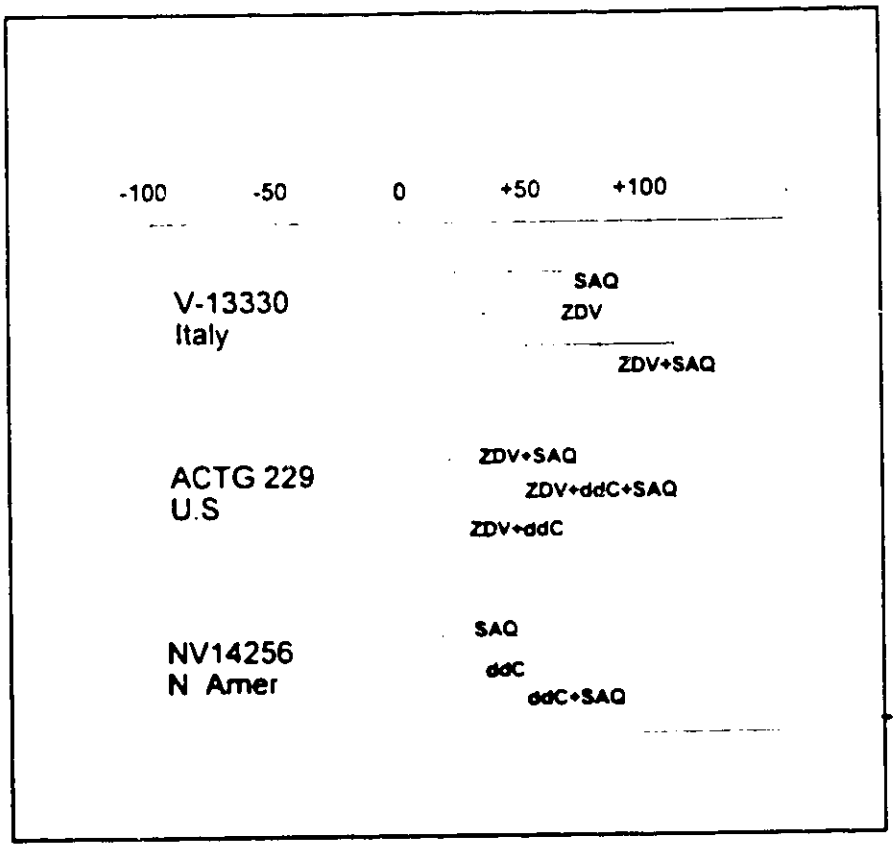
The activity of SAQ in combination with nucleoside analogues was demonstrated in three randomized, double-blind trials. A summary of mean changes in CD4 counts and HIV-RNA from baseline averaged over 16 weeks are graphically dismayed for the treatment arms of interest in Figures 10 and 11, respectively. Bars represent 95% confidence intervals around the mean.

CD4 cell counts

For naive patients (Italian study), treatment with ZDV+SAQ produced greater mean increases in CD4 cells than the control treatment, ZDV. Since the number of patients

per treatment arm in this study was small and the variability of CD4 measurements was substantial, confidence intervals are large and overlap. For ZDV experienced patients, the triple combination (ZDV+ddC+SAQ) produced greater increases in CD4 counts than the control treatment, ZDV+ddC. In ZDV experienced individuals in study NV14256, patients randomized to ddC+SAQ had greater mean CD4 increases (DAVG-16) than the control treatment ddC. For the latter two trials both comparisons of interest achieved statistical significance.

Fig. 10. Saquinavir combination regimens. Mean changes in CD4 from baseline (DAVG-16) compared to controls (with 95% confidence intervals).



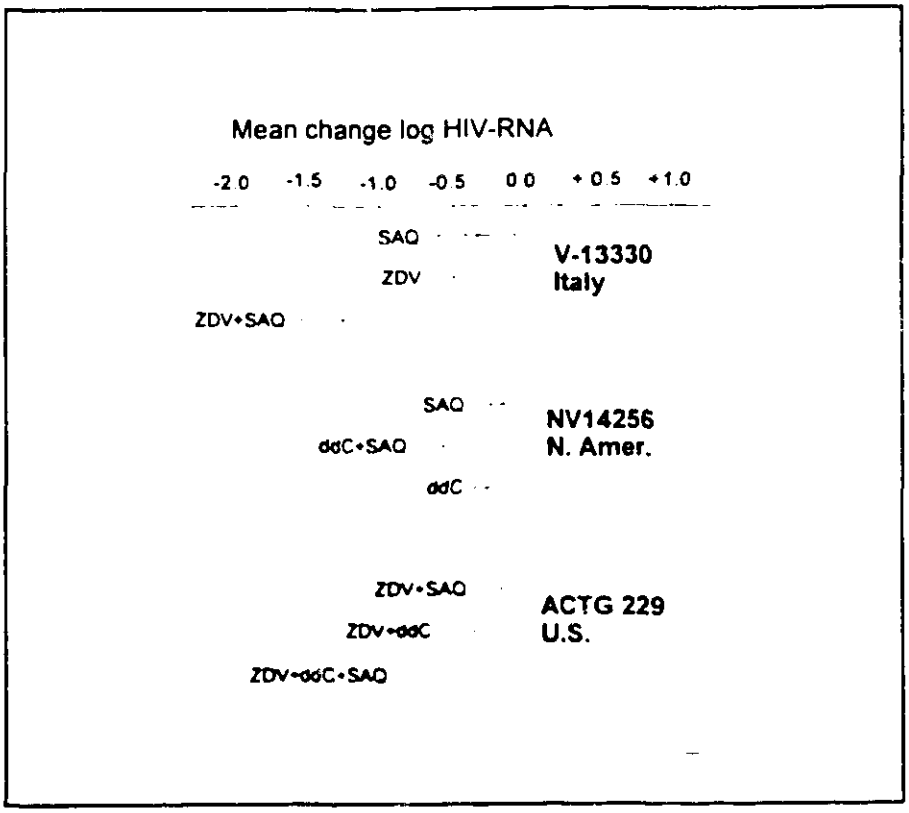
Source: Kazem Kazempour, SAS applications

HIV-RNA

For antiretroviral naive patients (Italian study), ZDV+SAQ produced greater mean HIV-RNA reductions compared to the control treatment, ZDV. For ZDV-experienced patients in ACTG 229, the triple combination produced greater RNA reductions from baseline than ZDV+ddC. In the North American study (NV14256), ddC +SAQ produced greater RNA reductions than the control treatment ddC. All of the above comparisons achieved statistical significance (see Fig. 11). At present it is unknown

if these increases in CD4 counts or decreases in plasma RNA copy numbers confer clinical benefit.

Fig. 11. Mean Changes in HIV-RNA with 95% confidence intervals. Saquinavir combinations compared to controls.



Source Kazem Kazempour, SAS applications

Additional comparisons of combination therapy

A comparison of surrogate marker data across studies demonstrates that the activity of SAQ combination regimens was attributable to SAQ plus a nucleoside analogue that patients had not previously received. Two examples illustrate this point. The first example is a comparison of the activity of SAQ plus ZDV (in patients who were ZDV-naive) versus the same regimen in patients who had received prolonged treatment with ZDV (in other words, adding SAQ to a continued regimen of ZDV). These two groups of patients with very similar baseline characteristics, except for prior ZDV use, had different surrogate marker responses to ZDV+SAQ treatment. Table 87 shows the baseline characteristics for naive patients receiving ZDV+SAQ in the Italian study and for ZDV-experienced patients receiving the same regimen in ACTG 229. Figures 12 and 13 show the CD4 and RNA changes respectively for patients receiving ZDV+SAQ in two studies. Both RNA reductions and CD4

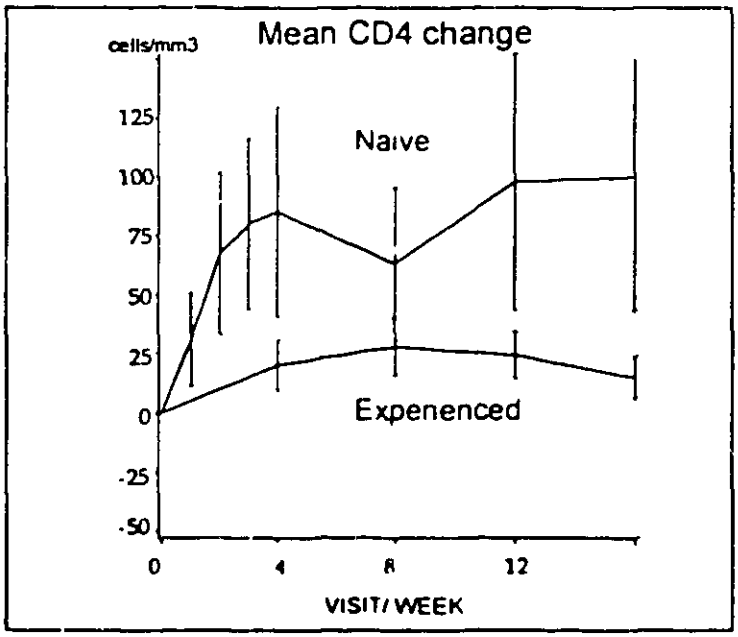
increases were greater among patients who had not previously received ZDV.

Table 87. Baseline characteristics for two treatment arms in ACTG 229 and NV14256.

Baseline	ACTG 229 Triple ZDV+ddC+SAQ	NV14256 ddC+SAQ
CD4 (median)	146	156
log_{10}RNA (median)	4.8	5.3
Prior ZDV use (median days)	647	435

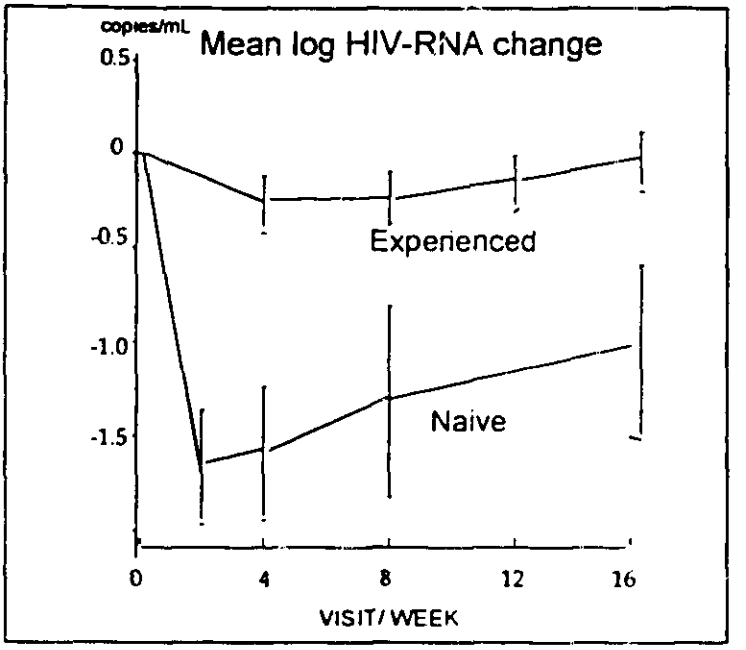
Source: excerpted from Tables 6, 26, 27 above.

Fig. 12. Mean changes in CD4 from baseline in patients receiving ZDV+SAQ in studies V-13330 and ACTG 229.



Source: Kazem Kazempour SAS applications

Fig. 13. Mean changes in HIV-RNA in patients receiving ZDV+SAQ in studies V-13330 and ACTG 229.



Source: Kazem Kazempour SAS applications.

The second example is a situation in which continuing ZDV in an triple combination with ddC+SAQ offered no additional activity compared to a double combination of ddC+SAQ. A comparison of the triple combination ZDV+ddC+SAQ in ACTG 229 and the double combination ddC+SAQ in NV14256 illustrates this point. Table 88 show that patients in both studies had similar baseline prognostic characteristics. Both study arms had prolonged prior treatment with ZDV. Figures 14 and 15 show the CD4 changes and RNA changes from baseline, respectively. In both cases, the plots of these changes are overlapping, indicating a nearly identical response. Therefor, continuing ZDV appeared to add no additional acticity to the ddC plus SAQ combination.

Table 88. Baseline characteristic of patients receiving ZDV+SAQ in two studies.

Baseline	ACTG 229 ZDV-experienced	V-13330 Naive
CD4 (median)	155	172
log_{10}RNA (median)	4.9	5.3
Prior ZDV use (median days)	717	0

Source: excerpted from Tables 6, 44, 49 above

Fig. 14. **Mean changes in CD4 from baseline for triple combination vs. double combination in studies ACTG 229 and NV14256, respectively.**

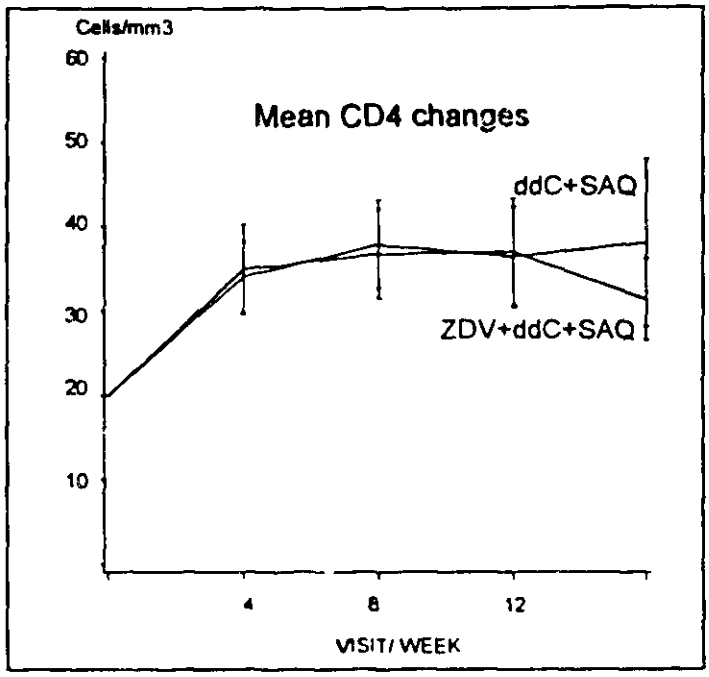
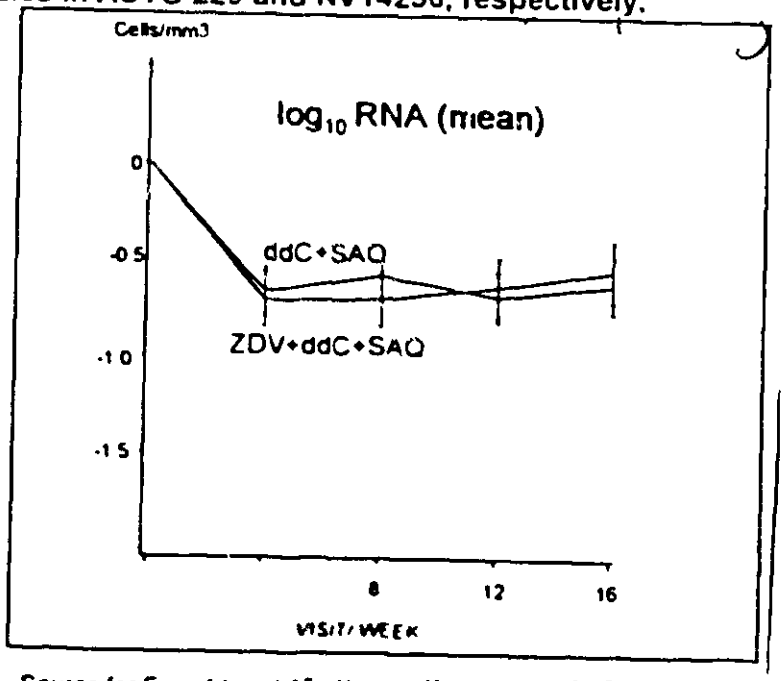


Fig. 15. **Mean Changes in HIV-RNA for triple combination vs. double combination in two studies in ACTG 229 and NV14256, respectively.**

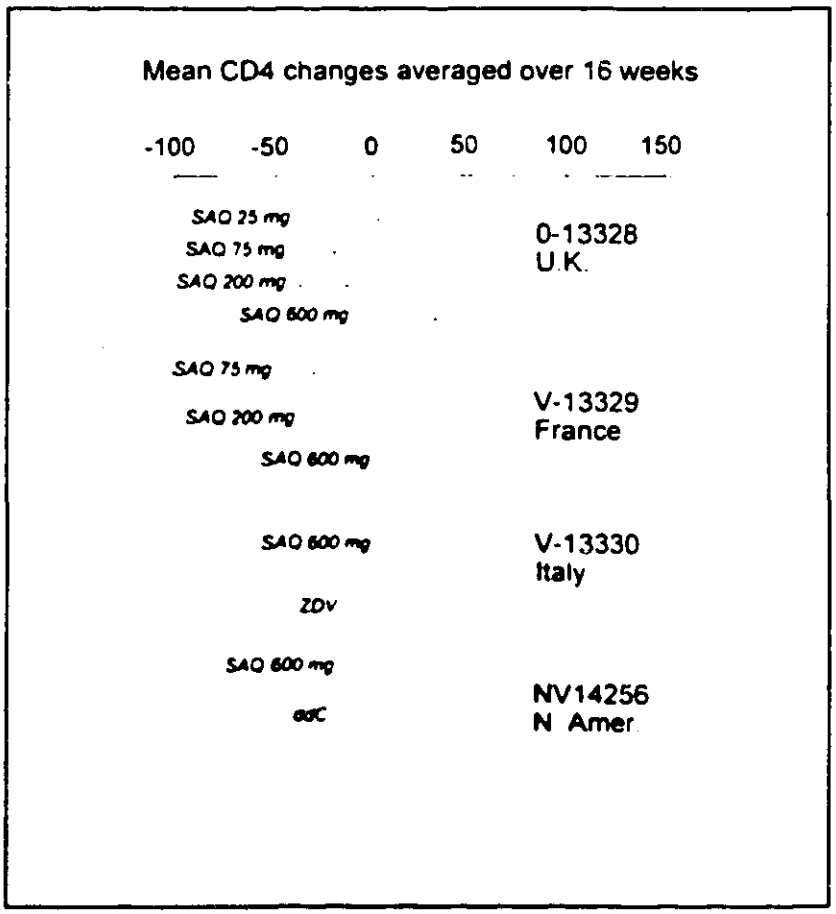


Source for Figs 14 and 15 Kazem Kazempour, SAS applications.

9.3 SAQ Monotherapy

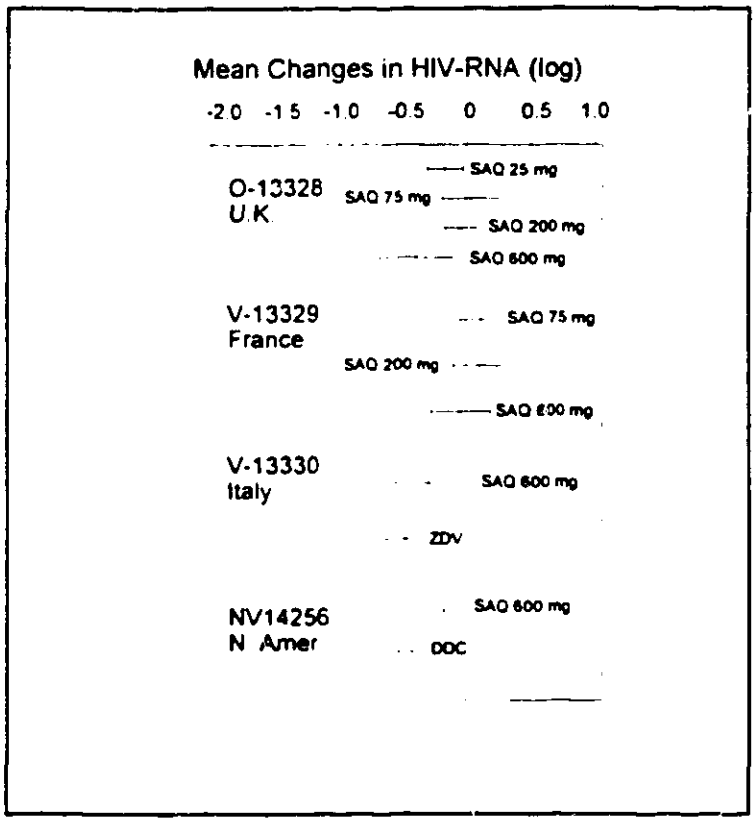
In clinical trials SAQ 600 mg tid monotherapy was compared to ZDV monotherapy, ddC monotherapy and also lower doses of SAQ (25, 75 and 200 mg tid) in dose-ranging trials. Figures 16 and 17 show mean CD4 changes and HIV-RNA changes (DAVG-16), respectively, for SAQ monotherapy arms in 4 clinical trials that included SAQ monotherapy arms.

Fig. 16. Mean CD4 Changes averaged over 16 weeks. Monotherapy Comparisons in 4 trials.



Source: Kazem Kazempour SAS applications

Fig. 17. Mean changes in HIV-RNA (log) averaged over 16 weeks. Monotherapy comparisons in 4 trials.



Source: Kazem Kazempour, SAS applications

SAQ 600 mg tid compared to lower doses

As stated in section 9.1 above, statistically there was no apparent dose response for SAQ at doses less than 600 mg tid. The 600 mg tid dose appeared to be the lowest dose with discernible activity. In the two dose ranging monotherapy studies (O-13328, U.K. and V-13329, France), mean CD4 changes for SAQ 600 mg tid were 33 cells/mm^3 (C.I. -12, 38) in ZDV naive individuals and 26 cells/mm^3 (C.I., 7, 45) in ZDV-experienced individuals. With respect to CD4 increases, SAQ 600 mg tid was statistically superior to lower doses only in the French study. These studies were designed for "proof-of-concept" and not with the intent of detecting signicant differences.

For changes in HIV-RNA, the 600 mg tid dose of SAQ generally produced greater reductions than lower doses, however the response was minimal and short-lived, returning to near baseline by 12 to 16 weeks. Mean change averaged over 16 weeks (DAVG-16) was -0.4 log copies/mL (C.I., -0.7, -0.1) in ZDV-naive patients and 0 (C.I., -0.6, 0.5) in ZDV-experienced patients.

SAQ monotherapy compared to nucleosides.
Two trials compared treatment with SAQ monotherapy to nucleoside monotherapy. In V-13330, SAQ 600 mg tid was compared to ZDV 200 mg tid in ZDV naive individuals. In NV14256, SAQ 600 mg tid was compared to ddC 0.75 mg tid in ZDV-experienced individuals. In both cases SAQ produced somewhat greater CD4 increases but less reduction in RNA than the monotherapy controls (see Table 88).

Table 88. Mean CD4 and HIV-RNA changes averaged over 16 weeks for SAQ monotherapy and nucleoside analogue controls in two studies.

Study	Regimen	CD4 Change (95% C.I.)	HIV-RNA change (95% C.I.)
V-13330	SAQ 600mg tid	44 (6, 83)	-0.3 (-0.7, 0)
	ZDV	34 (7, 74)	-0.5 (-0.9, -0.1)
NV14256	SAQ 600 mg bd	16 (5, 27)	-0.1 (-0.2, 0)
	ddC	6 (-5, 17)	-0.3 (-0.4, -0.2)

Source: excerpted from Tables 29, 33, 46, and 51.

9.4 Activity, Overall Conclusions

Initiating SAQ in combination with ZDV (in ZDV-naive patients) or with ddC in ZDV experienced patients produced greater activity than initiating ZDV or ddC as monotherapy respectively. In ACTG 229 the triple combination of ZDV+ddC+SAQ appeared to be no better than the ddC+SAQ combination in NV14256 for ZDV experienced patients. The activity of this triple combination in ZDV-naive patients is presently unknown but being studied in Roche's international study, SV14604. Therefore, it appears that SAQ/nucleoside combination regimens produce superior activity compared to the same regimens without SAQ. However, the concomitant nucleoside should be one to which the patient has not had substantial prior exposure. It may be reasonable to consider extrapolating the data for SAQ plus ZDV or ddC to use with other nucleosides, although, no data are available for evaluating the safety or activity of these other combinations.

The activity of SAQ as monotherapy appears to be less than that in combination with a nucleoside. SAQ 600 mg tid appeared to be more active than lower doses of SAQ based on CD4 changes in the French study, although, the mean RNA change averaged over 16 weeks for this dose group was zero in this study. It is difficult to assign a rank order for the activity of nucleosides compared to other available drugs. SAQ produced comparable to slightly better CD4 changes compared to ZDV and ddC but less reduction in plasma RNA.

10 Overview of Safety

The database closure date for the original NDA submission was Jan. 2, 1995. A safety update included 5 additional months of data up to a closure date of June 1, 1995. Table 89 shows the number of individuals receiving SAQ 600 mg tid as monotherapy or in combination with nucleoside analogues in controlled, comparative clinical trials submitted to support the proposed indications.

Table 89. Subjects receiving Saquinavir in clinical trials included in the original NDA submission.

	Studies	Number of Patients
Monotherapy	European studies, NV14256	210
SAQ + ddC	NV 14256	147
SAQ + ZDV	ACTG 229, V-13330	119
SAQ + ZDV + ddC	ACTG 229	98
Total	–	574

Source:

In addition to the subjects listed in table 89 above, Roche submitted safety data up to June 1, 1995, from several ongoing comparative trials, dose ranging trials and roll-over protocols including:

- EV 14757, an open label, dose-ranging study evaluating higher daily doses of saquinavir.
- SV14604, a large international phase 3 trial
- SV14788, a roll-over protocol for individuals discontinuing study drug in a comparative phase 3 trial
- NV14802 a roll-over protocol for those patients who had participated in ACTG 229.

The data available from the ongoing trials are still blinded (except for EV14757 which is open-label), and includes only deaths, serious adverse events and events leading to premature withdrawals. Preliminary safety data from these trials were discussed under section 8.3 "Other Studies". The number of patients included in the safety data base participating in the ongoing clinical trials is listed in Table 90.

Table 90. Safety data base from ongoing trials at time of data base closure (June 1995).

Protocol	Number included, as of June 1, 1995
NV 14256 Surrogate Analysis cohort (unblinded)	
ddC	145
SAQ	159
SAQ + ddC	147
NV 14256 Remaining patients (blinded data)	497
NV 14256 Discontinued arm (unblinded)	108
SV14604 (blinded)	1348
SV14788	208
NV14802	190
EV14757	
3600 mg/day	20
7200 mg/day	21
ICC 001	232

Source: Safety Update, pg 12

10.1 Significant Adverse Events

The types of adverse events that occurred among individuals receiving SAQ either alone or in combination with nucleoside analogues was consistent across trials. The most frequent adverse events excluding those termed as "asthenia" (which is a generalized feeling of fatigue) were events categorized under the gastrointestinal system. Diarrhea, abdominal pain, abdominal discomfort, and nausea were the most frequent events associated with SAQ or SAQ containing regimens (excluding events known to be associated with ddC or ZDV). The only specific adverse event type that appeared to be more frequent on SAQ-containing arms was diarrhea. In study ACTG 229, diarrhea of any intensity and at least remotely related occurred in 46% on of subjects on ZDV+SAQ and 33% receiving ZDV+ddC+SAQ compared to 28% receiving ZDV+ddC. In NV14256, diarrhea occurred in 16% of individuals on SAQ, 12% of individuals on ddC+SAQ and 4% on ddC alone. In NV14256, all of the cases of diarrhea were either mild or moderate, there were no cases of severe or life-threatening diarrhea, except one occurring in the ddC group. Diarrhea also appeared to be more frequent at higher doses in the Stanford study, EV14757. The percentages of patients experiencing diarrhea in this study were 45% for those receiving 3600 mg/day and 48% for those receiving 7200 mg/day. This can be compared to a frequency of 16% diarrhea in a group of naive patients receiving 1800 mg/day pooled from two European studies (0-13328 and V-13330).

The number of events and the percentage of individuals experiencing at least one event (considered to be at least remotely related) was much higher in ACTG 229 than in

NV14256. In Roche's integrated safety summary, they hypothesize a possible reason for this. They state that the ACTG 229 investigators may have had more careful reporting practices during this study since this was the first U.S. trial studying SAQ. However, there were differences in the designs and implementation of these two trials which could also explain a higher frequency of events in ACTG 229 compared to NV14256. First, the total duration of study treatment was longer in ACTG 229 compared to NV14256 and second, all patients in ACTG 229 received ZDV in addition to SAQ, ddC or ddC+SAQ (i.e., ZDV was added to every treatment arm).

Safety of doses higher than 600 mg tid (the proposed marketing dose)are being studied in trial EV14757. The type of events seen at lower doses are also seen at these higher doses with diarrhea, abdominal discomfort and nausea among the most common. Asthenia, headache, dizziness, cough, and rhinitis were also noted. See Table 83 for a more complete list.

10.1.1 Deaths

Table 91 lists the number of deaths and serious adverse events that have occurred in completed and ongoing trials. Among the 16 deaths occurring in the 5 trials submitted in this NDA to support the proposed indications, only one death was considered to be related to study treatment: one patient receiving ddC in NV14256 died from congestive heart failure and cardiomyopathy considered to be remotely related to treatment. Several other deaths have occurred in trials that are ongoing and remain blinded. Please refer back to the respective study reviews for details regarding serious adverse events

Table 91. Number of Deaths and Serious Adverse Events in completed and ongoing trials.

PROTOCOL	DEATHS (total no. of participants) *up to June 1995*	SERIOUS AEs (total no. of participants) *up to June 1995*
COMPLETED		
European Pooled V-13328, V-13329, V-13330	1 (202)	46 (202)
ACTG 229/NV14255	5 (302)	43 (302)
ONGOING		
NV 14256 (surrogate analysis cohort)	12 (451)	57 (451)
NV 14256 (remainder)	3 (497)	29 (497)
EV 14757	0 (41)	1 (41)
SV14604	4 (1348)	75 (1348)
SV14788	4 (208)	19 (208)
NV14802	3 (190)	2 (190)

Source NDA Safety Update, Vol 2, pg 2 and Tables 18 and 19 on pg 105.

10.1.2 10-Day Safety reports

Table 92 is a list of patients who developed adverse events requiring 10-day safety reports. The patient who experienced hemolytic anemia was also taking dapsone, which is known to be associated with this adverse event. The patient who was diagnosed with Steven's Johnson syndrome was also taking rifabutin and dapsone. Dapsone has been associated with severe skin rashes, including toxic epiermal necrolysis.

Table 92. 10 Day Reports

Protocol/Treatment	Event	Relationship
ACTG 229/SAQ + ZDV	confusion, ataxia and weakness	possible
ACTG 229/SAQ + ZDV	acute myeloblastic leukemia	remote
NV14256	hemolytic anemia	remote*
NV14256/SV14788	Stevens-Johnson syndrome	possible
NV14256	rash elevated transaminase levels and hepatomegally	probable possible
SV14604/(blinded)	attempted suicide by drug overdose with amitryptiline/temazepam	possible
SV14604	seizure in patient with previously controlled seizure disorder	possible
SV14604	thrombophlebitis	possible
SV14604	elevated transaminases, bilirubin and alkaline phosphatase	possible
SV14788	worsening headaches/increased CSF pressure	possible

Source Integrated Safety Summary (W144'973) pp 226-227 and Safety Update, Vol. 2, pp. 98-99.
*The sponsor states that this was more likely related to concomitant use of dapsone

10.2 Other Safety Findings

10.2.1 Laboratory Findings

Several patients interrupted or discontinued SAQ secondary to elevated transaminases or had transaminase elevations that were considered marked or serious These are summarized as follows

In ACTG 229, 3 patients on SAQ combinations had elevations of transaminases as part of a serious adverse event One of these individuals had a grade 2 elevation at baseline and one was rechallenged with SAQ without recurrence. Four patients in ACTG 229 had marked elevations of ALT (SGPT): 1 receiving ZDV+ddC, 0 receiving ZDV+SAQ, and 4 receiving ZDV+ddC+SAQ.

In NV14256, One patient on ddC and two on SAQ monotherapy discontinued

treatment after developing grade 3 or 4 liver enzyme abnormalities. One patient discontinued ddC because of elevated liver function tests that was attributed to alcohol. Although a few patients on the SAQ arm discontinued drug due to elevation of transaminases, less than 1% of individuals had marked elevations of AST or ALT compared to approximately 3% of patients on ddC monotherapy.

In V-13330, 3 patients on SAQ 600 mg and 3 patients on ZDV+SAQ 600 mg tid had marked elevations of ALT. Two patients on ZDV+SAQ 200 mg tid and one patient on ZDV had marked ALT elevations.

In studies 0-13228 and V-13329, there were no serious adverse events or dose-limiting events involving elevation of transaminases. No patients receiving SAQ 600 mg tid in these trials experienced marked elevations of transaminases.

Although several patients had dose-limiting elevation in transaminases, the relationship of these changes to SAQ administration was not always clear. Some of these patients had underlying hepatic disease (chronic hepatitis B), others were on concomitant medications known to be associated with transaminase elevation and some did not have recurrence of transaminase elevation upon rechallenge. There did not appear to be a predominance of transaminase elevations among patients receiving SAQ in the two larger data bases (ACTG229 and NV 14256). Table 93 is an excerpt of the laboratory abnormalities table that is included in the INVIRASE label

Table 93. Percentage of patients with marked transaminase elevations in NV14256 and ACTG 229

LAB	NV 14256			NV14255/ACTG229		
	ddC	*SAQ*	*ddC+SAQ*	*ZDV+ddC*	*ZDV+SAQ*	*ZDV+ddC+SAQ*
SGOT (AST)	3	<1	<1	0	2	2
SGPT (ALT)	3	<1	<1	1	0	3

Source excerpted from Tables 33 and 41 above

10.2.3 Overdosage exposure

In clinical trials, two patients have taken acute overdoses of SAQ. A 30 year old male receiving SAQ 25 mg tid in trial 0-13328 was admitted to hospital after taking 63 capsules of saquinavir and drinking alcohol all day. The patient underwent gastric lavage approximately 4 hours after drug ingestion, and made a complete recovery. It is estimated that this patient ingested 1575 mg of SAQ. This single dose is only slightly higher than the highest dose in the Merigan study, in which patients are taking 1200 mg q4 hrs.

Patient no 109 (0-13328, 600 mg SAQ), a 22 year old male, was admitted to a hospital at the end of week 10, having taken 40 capsules of saquinavir after

heavy alcohol intake. The patient was given an emetic approximately 2 hours after drug ingestion, vomited almost immediately, and made a complete recovery.

10.2.4 Human Reproduction Data

In the SAQ clinical trials program, three pregnancies occurred in patients taking saquinavir, a fourth pregnancy occurred in a patient taking ZDV + ddC, and a fifth occurred in a patient in study SV14604 (treatment still blinded). Of the three pregnancies occurring in women known to be taking SAQ, 2 chose elective termination of pregnancy, and the third (on SAQ 600 mg tid) had a spontaneous abortion five weeks after stopping study medication. One of the pregnancies was the result of "inadequate contraceptive cover" and another the result of condom breakage.

Therefore, there are no cases of live births after exposure of women to SAQ during pregnancy.

10.3 Relative Safety: Risks versus Benefit

Although it is hard to rank the activity of SAQ relative to that of ZDV or ddC, SAQ appears to be better tolerated and associated with fewer dose-limiting toxicites than either ddC or ZDV. Adverse events commonly associated with ddC or ZDV (such as neuropathy and stomatitis for ddC and nausea, headache and anemia for ZDV) were not frequent toxicities among individuals receiving SAQ.

With respect to adverse events that caused premature withdrawal from treatment, 45 patients in the NV14256 surrogate analysis safety population withdrew from treatment due to protocol treatment or non-protocol treatment toxicity. Of those patients, there were 19 withdrawals in the ddC group and 12 in the SAQ group (of which only 3 were protocol treatment toxicities, see Table 94). The most common cause of premature withdrawal was peripheral neuropathy. Table 95 compares the frequency of adverse events that are commonly attributed to ddC among patients receiving either ddC or SAQ in study NV14256. Peripheral neuropathy and buccal mucosal ulceration occurred less frequently among patients receiving SAQ.

Table 94. Number of Patients Withdrawing Prematurely due to Protocol or Non-Protocol Treatment Toxicity in study NV14256.

Treatment	ddC n = 145	SAQ 600 mg tid n = 159
Protocol treatment toxicity	12	3
Non-protocol treatment* toxicity	7	9
Total patients withdrawing from treatment prematurely	19	12

Source: CANDA SR for NV14256; W144'954, Table 20, pg. 70.
* Non-protocol treatment toxicity is not defined by the protocol, but by the patient requesting treatment discontinuation.

Table 95. Study NV14256: Number (percentage) of patients with AEs (all intensities excluding unrelated) that are known to be associated with ddC in patients.

ADVERSE EVENT	ddC *N=145*	SAQ *N=159*
Total Patients with at least one AE	76 (52)	75 (47)
Total number AEs	205	161
Buccal mucosal ulceration	22 (15)	10 (6)
Peripheral neuropathy	19 (13)	7 (4)
Numbness extremities	10 (7)	4 (3)
Paraesthesia	7 (5)	2 (1)

Source: CANDA SR for NV14256 W144'954, excerpted from Table 17, pg 52-62.and NDA Safety update pages 48-59

There were too few patients in V-13330, the only study directly comparing ZDV monotherapy with SAQ monotherapy, to make conclusions regarding the number of treatment withdrawals among arms in this study However, adverse events in any body system or those commonly associated with ZDV (such as, nausea, vomiting, abdominal pain, headache) were seen in fewer patients initiating SAQ than ZDV (see Table 96).

Table 96. Number of patients (percentage) experiencing adverse events (all intensities and at least remotely related) commonly associated with ZDV in V-13330.

EVENT	SAQ N=13	ZDV N=17
ALL BODY SYSTEMS	10 (53)	16 (94)
Nausea	2 (11)	10 (59)
Abdominal Pain	1 (5)	4 (24)
Vomiting	1 (5)	2 (12)
Diarrhea	2 (11)	1 (6)
Headache	3 (16)	5 (29)

Source: CANDA FSR for V-13330, W144'918, excerpted from Table 33, pp 84-88.

There were too few patients in V-13330 to compare the frequency of marked anemia among patients receiving either ZDV or SAQ monotherapy. However, the frequency of marked anemia among patients receiving SAQ monotherapy in study NV14256 was low, <1% over the 16 week study period.

11 Labeling Review

During the NDA review process, FDA and Roche discussed the content and specific wording of the SAQ label. We concur with the final print label submitted by Roche on 12/7/95. The following labeling review includes selected discussions of pertinent issues that arose during the review process. Only label headings/subheadings that required significant modifications from Roche's initial proposal are included.

11.1 Clinical Pharmacology/Microbiology

FDA recommended that Roche include specific information regarding the occurrence of genotypic and phenotypic changes associated with the use of INVIRASE. FDA, the FDA antiviral advisory committee, and members of the HIV scientific and lay community expressed concern over the potential development of cross-resistance to other protease inhibitors in development. After review of the SAQ resistance data by FDA microbiologists and by Dr. Douglas Mayers (who served both as an FDA consultant and presented at the antiviral advisory committee), it is inconclusive what effect the use of SAQ will have on the subsequent activity of other protease inhibitors. Specifically, it is unknown whether patients who receive SAQ will develop HIV strains with reduced susceptibility to subsequent protease inhibitors. It is also unknown whether co-mutations to SAQ could play a role in facilitating a more rapid development of resistance to other protease inhibitors. However, there does not appear to be a high degree of overlap between SAQ and the other protease inhibitors (furthest along in development, specifically ABT-538 and MK-639) with respect to the mutations correlated with in vitro resistance. Given the amount of

uncertainty with respect to cross-resistance between protease inhibitors, we asked Roche to include the following paragraph under a subheading entitled, "Cross-resistance to other antiretrovirals":

> The potential for cross-resistance between protease inhibitors has not been fully explored. Therefore it is unknown what effect saquinavir therapy will have on the subsequent use of other protease inhibitors.

This section is also cross-referenced under the Precautions section of the label.

11.2 Indications and Usage

FDA review and advisory committee recommendations concurred that SAQ should be indicated for use in combination with a nucleoside analogue but not for use as monotherapy. Defining a succinct combination indication in the setting of several possible combinations and several patient subsets (ZDV-naive, ZDV experienced) is difficult. It is clear from the activity data as a whole that SAQ combinations are more active when used with a nucleoside analogue to which a patient has not had prolonged exposure. To describe the combination indication, FDA proposed a less directed indication for SAQ for use with nucleoside analogues followed by several caveats regarding the basis for the indication. The indication reads:

> INVIRASE in combination with nucleoside analogues is indicated for the treatment of advanced HIV infection in selected patients (See Description of Clinical Studies)" This indication is based on increases in CD4 cell counts in patients who initiated INVIRASE concomitantly with either ZDV (in previously untreated patients) or HIVID (in patients previously treated with prolonged zidovudine therapy) At present there are no results available from trials evaluating the activity of INVIRASE in combination with nucleoside analogues other than ZDV or HIVID There are also no results available form clinical trials confirming therapy with INVIRASE on HIV disease progression or survival.

This wording should give adequate instruction regarding the use of SAQ in combination with ZDV and or HIVID It does not rule out the use with other nucleosides but cautions the reader that there is no supporting data for use with other drugs such as ddI, or d4T This type of indication may permit less restrictive use by drug assistance organizations or insurance, pending information regarding the use of SAQ with other nucleosides Advisory committee members were in agreement with the wording of this indication

11.3 Warnings

To date all antiretroviral drugs with accelerated approvals have had a box warning alerting the reader to the fact that the approval was based on surrogate marker changes and not clinical endpoints We recommended that the label contain the following BOX WARNING

The indication for INVIRASE for the treatment of HIV infection is based on changes in surrogate markers At present there are no results from controlled clinical trial evaluating the effect of regimens containing saquinavir on survival or the clinical progression of HIV infection, such as the occurrence of opportunistic infections or malignancies.

11.4 Description of Clinical Studies

In this section we asked Roche to emphasize the point that the primary basis for accelerated approval of SAQ was changes in surrogate markers. Since the clinical relevance of changes in HIV-RNA is currently unknown, this data will be considered supportive and presented in summary form after the description of CD4 changes. Roche described the CD4 changes for each of the three combination trials and included plots of mean CD4 changes from baseline over the study analysis periods (16 weeks for V-13330 and NV14256 and 24 weeks for ACTG 229). HIV-RNA changes are summarized (after the description of CD4 changes) in one comparative table for all three studies. To avoid using the DAVG metric in the label, we decided to report log transformed RNA change at the end of the analysis period (16 or 24 weeks). The mean change at these time points corresponded quite well to the mean reduction averaged over 16 weeks (DAVG metric). It also provides the reader with a better illustration of the durability of RNA response. A caveat regarding the use of RNA changes for predicting clinical response is also included in the statement, "The clinical significance of changes in HIV-RNA measurements have not been established." At present, this laboratory measure is available on an experimental basis to monitor antiviral activity in clinical trials.

11.5 Precautions

Under this section we added a subtitle entitled Resistance/Cross resistance. The reader is referred to the Microbiology section to become informed about issue regarding potential cross-resistance

11.5.1 Information for patients

We recommended that Roche include a clear statement directing patients to take SAQ within two hours after a full meal The following statement is included: "When INVIRASE is taken without food, plasma concentrations of saquinavir are substantially reduced and may result in no antiviral activity."

11.5.2 Drug interactions

Since saquinavir is metabolized primarily via the cytochrome p450 enzyme 3A, there are several potential drug interactions Roche has conducted pharmacokinetic studies with rifampin, rifabutin and ketoconazole. Rifampin and rifabutin are metabolic inducers and have been shown to reduce saquinavir plasma concentrations by 80% and 40% respectively. Given that the 600 mg tid dose is near the activity threshold for saquinavir, any reduction in exposure is crucial We recommended that Roche include statements in the label suggesting

that physicians not use rifampin concomitantly with saquinavir and consider alternative agents for rifabutin (when possible) when a patient is taking SAQ. Other drugs that induce cytochrome p450 (e.g., phenobarbitol, phenytoin, dexamethasone, and carbamazepine) are included in this precaution regarding potential reduction in saquinavir plasma concentrations.

Although Roche has not shown that saquinavir is a potent p450 3a inhibitor, it may compete with other drugs metabolized via the same pathway. It is well known that co-administration of terfenadine or astemizole with drugs known to be potent inhibitors of the p4503A pathway (i.e., ketoconazole, itraconazole, etc.) may lead to elevated plasma concentrations of terfenadine astemizole, which may in turn prolong QT interval leading to rare cases of serious cardiovascular events. The label includes this information with the following, "Although saquinavir is not a potent inhibitor of Cyp4503a, pharmacokinetic interaction studies with INVIRASE and terfenadine or astemizole have not been conducted, therefore, physicians should use alternatives to terfenadine or astemizole when a patient taking INVIRASE requires antihistamines." In addition other compounds that are substrates of cytochrome p4503A are mentioned.

11.6 Pediatric use

Unfortunately, there is no pediatric formulation for SAQ and studies have not been conducted in patients <18 years of age. Therefore no pediatric use statement can be made

11.7 Adverse Reactions

We did not differ with Roche's assessment of the safety data.

11.8 Dosage and Administration

Under the subheading "Dose Adjustment for combination therapy with INVIRASE," we recommended not including a dose reduction scheme for SAQ since lower doses have not shown antiviral activity. For adverse events that develop in patients taking SAQ (and are not related to other drugs), SAQ should be interrupted When the toxicity resolves, patient may be rechallenged at full doses

12 Conclusions

Roche has requested accelerated approval of INVIRASE based on changes in surrogate markers For the indication of INVIRASE 600 mg tid in combination with nucleoside analogues, Roche has demonstrated superior CD4 increases and plasma HIV-RNA reductions in patients receiving INVIRASE containing combination regimens compared to controls in three randomized, double-blind trials. In treatment naive patients, ZDV+SAQ 600 mg tid produced superior antiviral activity compared to ZDV In ZDV-experienced patients, ddC+SAQ 600 mg tid produced superior antiviral activity compared to ddC In a third trial, ZDV+ddC+SAQ produced superior

antiviral activity to ZDV+ddC in ZDV experienced patients. When comparing data across studies, it is apparent that the activity of INVIRASE containing combinations is attributable to INVIRASE and a nucleoside to which patients have not had previous exposure. This constitutes important prescribing information for the clinician and patient.

The safety of INVIRASE 600 mg tid in combination with ZDV and/or ddC has also been established. INVIRASE does not appear to alter the pattern, frequency, or severity of adverse events known to be associated with ZDV and/or ddC. Diarrhea occurred more frequently in patients receiving higher doses of SAQ, beyond the recommended dose. Most cases of diarrhea were not dose limiting. A few patients withdrew from SAQ containing drug regimens secondary to elevation in transaminases. It is unclear in some cases if this was related to drug or underlying diseases, such as viral hepatitis, or other drugs.

There are no data available regarding the use of INVIRASE in combination with nucleosides other than ZDV and ddC. The label indication does not restrict the use of INVIRASE with other nucleosides but cautions the reader that there are no supporting data for use with other drugs such as ddI, or d4T. This type of indication may permit less restrictive use by third-party payers as more information regarding other combinations of nucleosides becomes available.

It is clear that the optimal dose of INVIRASE has not been established. Preliminary data included in the NDA suggest that twice and quadruple the current dose of INVIRASE may produce greater and more sustained increases in CD4 and reductions in RNA. Administering these doses with the current formulation is not logistically feasible, because of the large number of capsules that must be ingested to achieve higher exposures. Roche is currently working on a new formulation (soft gelatin capsule formulation) that may increase bioavailability and drug exposure.

Given the limitations of the current formulation with respect to optimal drug activity, concerns have been raised regarding the potential for INVIRASE to promote cross resistance to other protease inhibitors that are currently in development. At present the risk for cross-resistance is unknown, as was acknowledged by FDA consultant Douglas Mayes M.D. and members of the antiviral advisory committee. The potential for cross-resistance is clearly stated in the label.

The activity of INVIRASE as monotherapy, as demonstrated in clinical trials, was modest at best. SAQ 600 mg tid appeared to be superior to lower doses of SAQ with respect to CD4 increases. This was less apparent for changes in RNA. From the data, one is unable to rank the relative activity of SAQ in comparison to ZDV and ddC. For CD4 changes, SAQ appeared to be slightly more active but for reductions in HIV-RNA, SAQ appeared to be less active. In NV14256, SAQ produced statistically less mean RNA reductions than ddC.

13 Recommendations

Our analyses of the activity and safety data of the trials submitted support an accelerated approval of INVIRASE for use as combination therapy with nucleoside analogues in the treatment of advanced HIV infection. The activity of INVIRASE monotherapy, as demonstrated in these trials, do not adequately support the use of INVIRASE as monotherapy. A federal advisory committee panel met on Nov. 7, 1995, to give recommendations regarding the accelerated approval of INVIRASE. The committee recommended (in a vote of 7 for, 1 against) for the accelerated approval of INVIRASE for use in combination with nucleoside analogues in the treatment of selected patients with advanced HIV. They unanimously voted against accelerated approval for INVIRASE as single agent therapy in the treatment of HIV infection.

13.1 Accelerated Approval Commitments

As required by Subpart H regulations, Roche has committed to providing clinical confirmatory data for the use of INVIRASE in combination with nucleoside analogues. We concur with the design of the two protocols that will be used to provide clinical confirmation. We also concur with Roche's decision tree for proposed actions in response to possible study outcomes. Details of the primary statistical analyses will be discussed in future communications with Roche. The two ongoing clinical studies, designed and powered to detect differences in HIV disease progression and death, are:

1) NV14256

This study has three treatment arms, comparing ddC+SAQ to ddC alone and SAQ alone. Primary endpoints are HIV disease progression (CDC 1993 definition of AIDS defining events) and death. It is scheduled to be completed during the first quarter of 1996. The primary comparison will be ddC+SAQ versus ddC alone.

Roche has submitted a decision tree regarding actions that will be taken based on potential outcomes. If the combination is statistically significantly superior to ddC monotherapy, Roche plans to submit an application for traditional approval. If this comparison results in a positive trend for the superiority of the combination compared to ddC alone, then Roche will continue marketing INVIRASE under accelerated approval, while awaiting data from the second clinical trial. If there is not difference between the double combination and ddC or if there is a negative trend, Roche and the FDA will consider presenting the data to an advisory committee and/or revising the current label. If the combination is significantly worse, the accelerated approval will be withdrawn.

2) SV14604

This study will evaluate clinical endpoints of HIV progression or death in naive

patients (defined as less than 4 months of prior ZDV). Originally this study had 4 study arms:
ZDV
ZDV+ddC
ZDV+SAQ
ZDV+ddC+SAQ

After the release of preliminary data from studies ACTG 175 and the Delta trial indicating the superiority of combination treatment compared to ZDV monotherapy in naive patients, Roche stopped enrolling patients into the ZDV monotherapy arm and switched the individuals already randomized to the triple combination.

The primary comparison will be difference in HIV disease progression and death between the triple combination and ZDV+ddC. If this comparison shows statistically significant superiority, then clinical confirmation will be established.

13.2 Phase 4 Commitments

We concur with Roche's proposed phase 4 commitments. Roche was asked to include time frames for completions of products already started or in the design phase. Commitments include

- Development of a pediatric formulation
- PK interaction studies and surrogate marker studies with ritonavir and other protease inhibitors
- Surrogate marker study with ZDV and Epivir
- Exploration of cross-resistance with other protease inhibitors
- Terfenadine pharmacokinetic interaction study

Roche also plans to develop of a soft gelatin formulation of INVIRASE, with increased bioavailability. This includes exploration of higher doses/exposures of SAQ

13.3 Labeling

We concur with the content and format of Roche's final print label for INVIRASE submitted on 12/7/95. Please refer to the labeling review for specific labeling issues discussed during the review process

Jeffrey S. Murray, M.D.
Medical Officer

Concurrences:
HFD-530/Feigal
HFD-530/Freeman
FD-530/Gitterman

cc:
HFD-530/orig NDA
HFD-530/Division File
HFD-530/Murray
HFD-530/Sahajwalla
HFD-530/jenkins
HFD-530/Farrelly
HFD-530/Liu
HFD-530/Batulla
HFD-530/Lard
HFD-530/Iaconos-Connors
HFD-530/K.M. Wu
HFD-530/Kinsey
HFD-715/Kammerman

Appendices

Appendix I

CENTER / SUBCENTERS CENTER NO.

1) University of Rochester, Rochester, NY / 1101
   SUNY Health Science Center, Syracuse, NY / 1102
   Erie County Medical Center, Buffalo, NY 1103
2) Stanford University, Stanford, CA / 501
   Kaiser Permanante Medical Center, San Francisco, CA / 502
   AIDS Community Research Consortium, Redwood City, CA / 506
   Santa Clara Medical Center, Santa Clara, CA / 506
   San Mateo Medical Center, San Mateo, CA 506
3) Ohio State University, Columbus, OH 2301
4) University of Washington, Seattle, WA 1401
5) University of Alabama, Birmingham, AL 5801
6) University of Colorado, Denver,CO 6101
7) University of Texas, Galveston, TX 6301
8) University of Pennsylvania, Philadelphia, PA / 6201
   Thomas Jefferson University, Philadelphia, PA 6202
9) Northwestern University, Chicago, IL / 2701
   Rush Presbyterian - St. Luke's Medical Center, Chicago, IL / 2702
   Cook County Hospital, Chicago, IL 2705
10) New York University, Bellevue Hospital, NY, NY 401

APPENDIX II

Listing of AIDS-Defining Events by Patient

CENTER	PID	AIDS-DEFINING EVENT
12838	40	PCP
	46	CRYPTOCOCCAL MENINGITIS
12909	21	CRYPTOSPORIDIOSIS
	27	PNEUMOCYSTIS CARINII PNEUMONIA
	31	CRYPTOSPORIDIOSIS
	851	CRYPTOSPORIDIOSIS
	854	KAPOSI'S SARCOMA OF LUNG
	857	CRYPTOSPORIDIOSIS
12911	178	PCP
12912	495	RECURRENT HERPES ZOSTER
	497	CMV RETINITIS
	497	MAI
12916	980	KAPOSI'S SARCOMA
12919	375	CRYPTOSPORIDIAL DIARRHEA
	376	CRYPOSPORIDIAL DIARRHEA
12920	410	CMV COLITIS - RECURRENT
	412	CMV RETINITIS
	1245	CMV COLITIS
12921	94	ESOPHAGEAL CANDIDIASIS
12927	561	1A PC PNEUMONIA
	562	PCP
12957	676	KAPOSIS SARCOMA
14157	145	CRYPTOSPORIDIOSIS/DIARRHEA > 1 MONTH
14161	433	DISEMINATED ZOSTER - PRIMARILY VARICELLA
	434	PRESUMPTIVE PML
	1126	MYCOBACTERIUM TUBERCULOSIS
14162	210	PROGRESSIVE MULTILOCAL LEUKOERCEPHALPATHY
	1073	KAPOSI'S SARCOMA
	1080	KAPOSI'S SARCOMA
14163	506	KAPOSI'S SARCOMA
	506	PCP
14164	350	ESOPHAGEAL CANDIDIASIS
14167	705	CRYPTOCOCCAL MENINGITIS
14176	74	PRESUMPTIVE ESOPHAGEAL CANDIDIASIS
14291	776	ESOPHAGEAL CANDIDIASIS

Source W144954 pg 48 Table 14

Appendix III
Roche DAVG-24 analysis of cell-associated viremia as measured by PBMC co-culture

DAVG analysis: Summary of change in log(Viraemia Cell) over a 24 week period NV14255: Intent to treat Population

Treatment	SA ɔ/ZDV	SAQ/ZDV/DDC	ZDV/DDC
Mean			
n	97	97	100
Mean	0.0	-0.6	-0.3
Std Dev	0.7	0.8	0.8
Median	0.0	-0.6	-0.1

Source: CANDA

Note Roche DAVG definition used in calculations performed by the CANDA differs from FDA definition

Roche Pharmaceuticals
A Member of the Roche Group

[illegible] Roche Inc
340 [illegible] Street
[illegible] New [illegible] 07[illegible]

[illegible] (201) 812-3670
[illegible] (201) 812-3700

December 6, 1995

Food and Drug Administration
Division of Anti-Viral Drug Products
Center for Drug Evaluation and Research, HFD 240
Attn: Ms. Vikki Kinsey, CSO
9201 Corporate Boulevard
Rockville, Maryland 20850

Dear Ms. Kinsey

Re: NDA 20-628 - INVIRASE™ (saquinavir mesylate) Capsules
Agreement to Revise Package Insert

As agreed in our discussion today, Hoffmann-La Roche will make the following revisions to the final printed labeling delivered to the Agency on December 6, 1995.

The description of Table 1 will be revised to read as follows: Table 1. Frequency of Genotypic and Phenotypic Changes in Selected Patients Treated with Saquinavir. In addition, an additional footnote will read: For some patients genotypic and phenotypic changes were unrelated.

In response to a request from Dr. Murray, we have revised the demographic description for the safety update analysis population of NV14256 as follows: "median duration of treatment among the surrogate analysis cohort analyzed for safety (n=451) in NV14256 was 42 weeks."

A limited quantity of inserts identical to the FPL delivered on December 6, 1995 will be used in the packaging of those quantities of the drug to be shipped for the first month after the approval of INVIRASE. When this supply of inserts is exhausted, revised inserts reflecting the above noted changes will be used in packaging.

In addition, as agreed the revised insert will be used immediately for dissemination with all advertising and promotional materials

Should you have any questions, please feel free to contact the undersigned

Sincerely,

HOFFMANN-LA ROCHE INC.

Mary Ellen Mulligan

Mary Ellen Mulligan
Director, Therapeutic Group I
Drug Regulatory Affairs

MEM:jmd
12IAApl.doc
HLR No. 1995-2126

ORIGINAL

Roche Pharmaceuticals
A Member of the Roche Group

JAN 17 1996

(201) 812-3676
(201) 812-3554

January 16, 1996

NEW CORRESPONDENCE

Food and Drug Administration
Division of Anti-Viral Drug Products
Center for Drug Evaluation and Research, HFD-530
Attention: Document Control Room
9201 Corporate Boulevard
Rockville, Maryland 20850

Ladies and Gentlemen:

Re: NDA 20-628 - INVIRASE™ (saquinavir mesylate) Capsules
Patent Information Update

Pursuant to revised 21 USC 505(b), Hoffmann-La Roche Inc herewith submits updated patent information for INVIRASE (saquinavir mesylate) Capsules, approved under NDA 20-628

This submission updates the patent information previously filed to this NDA on August 31, 1995 Upon reconsideration, Item 9 "Patent Information" has been revised to state only United States Patent No 5,196 438, expiring November 19, 2010.

It is our understanding that the above information will be included in the next revision of the Approved Prescription Drug Products List (Orange Book).

Should you have any questions regarding this submission, please do not hesitate to contact the undersigned

Sincerely

HOFFMANN-LA ROCHE INC

Robin L Conrad
Manager
Drug Regulatory Affairs

RLC jmd
1IRPI doc
HLR No 1996-81

PATENT INFORMATION

1. Active Ingredient(s): Saquinavir

2. Strength(s): 200 mg capsules

3. Trade Name: INVIRASE®

4. Dosage Form and Route of Administration: Capsules, Oral

5. Applicant (Firm) Name: Hoffmann-La Roche Inc.

6. NDA Number: NDA 20-628

7. First Approval Date: Dec. 6, 1995

8. Exclusivity: First ANDA can not be submitted until 5 years after date of NDA approval.

9. Patent Information:

5,196,438	
Nov. 19, 2010**	
Drug Substance	
Hoffmann-La Roche Inc.	

While this submission was prepared in good faith, no warranty or guarantee is made regarding the accuracy or completeness of the information contained therein.

CONFIDENTIAL INFORMATION

**This date does not include any extension under 35 U.S.C. 156.

EXCLUSIVITY SUMMARY for NDA # 20-628 SUPPL # —

Trade Name INVIRASE Generic Name Saquinavir

Applicant Name Hoffman La-Roche HFD- 530

Approval Date Dec. 7, 1995?

PART I IS AN EXCLUSIVITY DETERMINATION NEEDED?

1. An exclusivity determination will be made for all original applications, but only for certain supplements. Complete Parts II and III of this Exclusivity Summary only if you answer "yes" to one or more of the following questions about the submission.

a) Is it an original NDA?

YES /✓/ NO /___/

b) Is it an effectiveness supplement?

YES /___/ NO /✓/

If yes, what type? (SE1, SE2, etc.) _____

c) Did it require the review of clinical data other than to support a safety claim or change in labeling related to safety? (If it required review only of bioavailability or bioequivalence data, answer "no.")

YES /✓/ NO /___/

If your answer is "no" because you believe the study is a bioavailability study and, therefore, not eligible for exclusivity, EXPLAIN why it is a bioavailability study, including your reasons for disagreeing with any arguments made by the applicant that the study was not simply a bioavailability study.

_____

_____

If it is a supplement requiring the review of clinical data but it is not an effectiveness supplement, describe the change or claim that is supported by the clinical data.

_____

_____

d) Did the applicant request exclusivity?

YES /___/ NO /✓/

If the answer to (d) is "yes," how many years of exclusivity did the applicant request?

_____

IF YOU HAVE ANSWERED "NO" TO ALL OF THE ABOVE QUESTIONS, GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

2. Has a product with the same active ingredient(s), dosage form, strength, route of administration, and dosing schedule previously been approved by FDA for the same use?

YES /___/ NO /✓/

If yes, NDA # _____ Drug Name _____

IF THE ANSWER TO QUESTION 2 IS "YES," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

3. Is this drug product or indication a DESI upgrade?

YES /___/ NO /✓/

IF THE ANSWER TO QUESTION 3 IS "YES," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8 (even if a study was required for the upgrade).

PART II FIVE-YEAR EXCLUSIVITY FOR NEW CHEMICAL ENTITIES
(Answer either #1 or #2, as appropriate)

1. Single active ingredient product.

Has FDA previously approved under section 505 of the Act any drug product containing the same active moiety as the drug under consideration? Answer "yes" if the active moiety (including other esterified forms, salts, complexes, chelates or clathrates) has been previously approved, but this particular form of the active moiety, e.g., this particular ester or salt (including salts with hydrogen or coordination bonding) or other non-covalent derivative (such as a complex, chelate, or clathrate) has not been approved. Answer "no" if the compound requires metabolic conversion (other than deesterification of an esterified form of the drug) to produce an already approved active moiety.

YES /___/ NO /✓/

If "yes," identify the approved drug product(s) containing the active moiety, and, if known, the NDA #(s).

NDA # _____ _____

NDA # _____ _____

NDA # _____ _____

2. Combination product.

If the product contains more than one active moiety (as defined in Part II, #1), has FDA previously approved an application under section 505 containing any one of the active moieties in the drug product? If, for example, the combination contains one never-before-approved active moiety and one previously approved active moiety, answer "yes." (An active moiety that is marketed under an OTC monograph, but that was never approved under an NDA, is considered not previously approved.)

YES /___/ NO /✓/

If "yes," identify the approved drug product(s) containing the active moiety, and, if known, the NDA #(s).

NDA # _____ _____

NDA # _____ _____

NDA # _____ _____

IF THE ANSWER TO QUESTION 1 OR 2 UNDER PART II IS "NO," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8. IF "YES," GO TO PART III.

PART III THREE-YEAR EXCLUSIVITY FOR NDA'S AND SUPPLEMENTS

To qualify for three years of exclusivity, an application or supplement must contain "reports of new clinical investigations (other than bioavailability studies) essential to the approval of the application and conducted or sponsored by the applicant." This section should be completed only if the answer to PART II, Question 1 or 2, was "yes."

1. Does the application contain reports of clinical investigations? (The Agency interprets "clinical investigations" to mean investigations conducted on humans other than bioavailability studies.) If the application contains clinical investigations only by virtue of a right of reference to clinical investigations in another application, answer "yes," then skip to question 3(a). If the answer to 3(a) is "yes" for any investigation referred to in another application, do not complete remainder of summary for that investigation.

YES /___/ NO /___/

IF "NO," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

2. A clinical investigation is "essential to the approval" if the Agency could not have approved the application or supplement without relying on that investigation. Thus, the investigation is not essential to the approval if 1) no clinical investigation is necessary to support the supplement or application in light of previously approved applications (i.e., information other than clinical trials, such as bioavailability data, would be sufficient to provide a basis for approval as an ANDA or 505(b)(2) application because of what is already known about a previously approved product), or 2) there are published reports of studies (other than those conducted or sponsored by the applicant) or other publicly available data that independently would have been sufficient to support approval of the application, without reference to the clinical investigation submitted in the application.

For the purposes of this section, studies comparing two products with the same ingredient(s) are considered to be bioavailability studies

(a) In light of previously approved applications, is a clinical investigation (either conducted by the applicant or available from some other source, including the published literature) necessary to support approval of the application or supplement?

YES /___/ NO /___/

If "no," state the basis for your conclusion that a clinical trial is not necessary for approval **AND GO DIRECTLY TO SIGNATURE BLOCK ON PAGE 8:**

_____

_____

(b) Did the applicant submit a list of published studies relevant to the safety and effectiveness of this drug product and a statement that the publicly available data would not independently support approval of the application?

YES /___/ NO /___/

(1) If the answer to 2(b) is "yes," do you personally know of any reason to disagree with the applicant's conclusion? If not applicable, answer NO.

YES /___/ NO /___/

If yes, explain: _____

_____

(2) If the answer to 2(b) is "no," are you aware of published studies not conducted or sponsored by the applicant or other publicly available data that could independently demonstrate the safety and effectiveness of this drug product?

YES /___/ NO /___/

If yes, explain: _____

_____

(c) If the answers to (b)(1) and (b)(2) were both "no," identify the clinical investigations submitted in the application that are essential to the approval:

Investigation #1, Study # _____

Investigation #2, Study # _____

Investigation #3, Study # _____

3. In addition to being essential, investigations must be "new" to support exclusivity. The agency interprets "new clinical investigation" to mean an investigation that 1) has not been relied on by the agency to demonstrate the effectiveness of a previously approved drug for any indication and 2) does not duplicate the results of another investigation that was relied on by the agency to demonstrate the effectiveness of a previously approved drug product, i.e., does not redemonstrate something the agency considers to have been demonstrated in an already approved application.

a) For each investigation identified as "essential to the approval," has the investigation been relied on by the agency to demonstrate the effectiveness of a previously approved drug product? (If the investigation was relied on only to support the safety of a previously approved drug, answer "no.")

Investigation #1 YES /___/ NO /___/

Investigation #2 YES /___/ NO /___/

Investigation #3 YES /___/ NO /___/

If you have answered "yes" for one or more investigations, identify each such investigation and the NDA in which each was relied upon:

NDA # _____ Study # _____

NDA # _____ Study # _____

NDA # _____ Study # _____

b) For each investigation identified as "essential to the approval," does the investigation duplicate the results of another investigation that was relied on by the agency to support the effectiveness of a previously approved drug product?

Investigation #1 YES /___/ NO /___/

Investigation #2 YES /___/ NO /___/

Investigation #3 YES /___/ NO /___/

If you have answered "yes" for one or more investigations, identify the NDA in which a similar investigation was relied on:

NDA # _____ Study # _____

NDA # _____ Study # _____

NDA # _____ Study # _____

c) If the answers to 3(a) and 3(b) are no, identify each "new" investigation in the application or supplement that is essential to the approval (i.e., the investigations listed in #2(c), less any that are not "new"):

Investigation #__, Study # _____

Investigation #__, Study # _____

Investigation #__, Study # _____

4. To be eligible for exclusivity, a new investigation that is essential to approval must also have been conducted or sponsored by the applicant. An investigation was "conducted or sponsored by" the applicant if, before or during the conduct of the investigation, 1) the applicant was the sponsor of the IND named in the form FDA 1571 filed with the Agency, or 2) the applicant (or its predecessor in interest) provided substantial support for the study. Ordinarily, substantial support will mean providing 50 percent or more of the cost of the study.

a) For each investigation identified in response to question 3(c): if the investigation was carried out under an IND, was the applicant identified on the FDA 1571 as the sponsor?

Investigation #1

IND # _____ YES /__/ NO /__/ Explain: _____

_____

Investigation #2

IND # _____ YES /__/ NO /__/ Explain: _____

_____

(b) For each investigation not carried out under an IND or for which the applicant was not identified as the sponsor, did the applicant certify that it or the applicant's predecessor in interest provided substantial support for the study

Investigation #1

YES /__/ Explain _____ NO /__/ Explain _____

_____ _____

_____ _____

Investigation #2

YES /___/ Explain _____ NO /___/ Explain _____

_____ _____

_____ _____

(c) Notwithstanding an answer of "yes" to (a) or (b), are there other reasons to believe that the applicant should not be credited with having "conducted or sponsored" the study? (Purchased studies may not be used as the basis for exclusivity. However, if all rights to the drug are purchased (not just studies on the drug), the applicant may be considered to have sponsored or conducted the studies sponsored or conducted by its predecessor in interest.)

YES /___/ NO /___/

If yes, explain: _____

_____

_____

_____

Nikki S. Kinney 11/21/95

Signature Date

Title: Consumer Safety Officer

David W. Fry 11-21-95

Signature of Division Director Date

cc: Original NDA Division File HFD-85 Mary Ann Holovac

NDA 20-628

Group Leaders Memorandum of NDA Application

Date: November 20, 1995

Product: INVIRASE (Saquinavir)
Sponsor: Hoffmann-LaRoche, Inc.

This application was submitted for approval of INVIRASE, the first of a class of antiretroviral agents that suppress HIV replication by inhibition of the HIV-viral protease enzyme. This is also the first NDA application for the treatment of HIV by a compound that acts through a mechanism other than the inhibition of viral reverse transcriptase, i.e., that is not a nucleoside analogue.

I have reviewed the superb Medical/Statistical review of this NDA by Drs. Murray and Kazempour and have little to add. I concur completely with their opinion, and similarly concur with the careful wording for the Indications and Usage section that Dr. Murray recommends be incorporated into the package insert. The decision for the approval of INVIRASE is particularly difficult; it seems virtually certain from the materials submitted that the dose of INVIRASE recommended for approval will be below an optimal therapeutic dose. There are also substantial concerns regarding the development of resistance which may render subsequent protease inhibitors (or improved formulations of INVIRASE) ineffective. However, despite these concerns, approval is warranted in the context of the very limited options available to HIV-infected persons. The sponsor has demonstrated that the drug is active in combination with nucleoside analogues (to which the patients are previously unexposed), even at the proposed dose. The sponsor has fulfilled the criteria for accelerated approval for this agent, and confirmatory clinical trials are in place. As reiterated in the medical/statistical review, the FDA Antiviral Advisory Committee endorsed approval of INVIRASE recognizing the limitations of the present dose and the potential for induction of viral resistance.

Recommendation: I wholly concur with the recommendation for approval of INVIRASE in combination with approved nucleoside analogues. The wording suggested by Drs. Murray/Kazempour is both appropriate and warranted.

Steven Gitterman, M.D., Ph.D.
Medical Officer/Team Leader

DRAFT

CLINICAL PHARMACOLOGY/ BIOPHARMACEUTICS REVIEW

NDA: 20,628
DRUG: Saquinavir capsules, 200 mg (INVIRASE™)
APPLICANT: Hoffman-La Roche
TYPE: NME

REVIEWERS: Janice B. Jenkins, Ph.D. & Chandrahas Sahajwalla, Ph.D.
SUBMISSION DATES: August 31; September 15, 21, 26, 27, 28, 29; October 4, 5, 9, 23, 24, 25, 26, 27; November 20, 27 and December 6, 1995
DRAFT REVIEW: 10/17/95
FINAL REVIEW:

BACKGROUND:

The Applicant is seeking approval of saquinavir (INVIRASE™) capsules, 200 mg. Saquinavir belongs to a class of compounds known as proteinase (protease) inhibitors, and is intended to be used in the treatment of HIV infection.

SYNOPSIS:

Healthy Volunteers

IV - Intravenous infusion (6 to 72 mg infused over 3 hours) of saquinavir in the fed state appeared to follow linear pharmacokinetics. Administration of i.v. saquinavir with food increased its clearance, probably due to a transient increase in hepatic blood flow after meals. Saquinavir distributes widely and has a volume of distribution of ~700 L. Saquinavir is highly protein bound (~98%) and the binding is linear over the range of 15-700 ng/mL.

ORAL - A ^{14}C-saquinavir study in healthy volunteers (I.V. and oral single dose) resulted in 85% to 89% of the radiolabelled dose being recovered in the urine and feces. Radioactivity recovered in the feces was 81% and 88% following iv. and oral administration, respectively. Following intravenous and oral administration 66% and 13% of the circulating radioactivity was attributed to the parent, respectively. Data suggest that about 20% to 30% of radiolabelled saquinavir reaches systemic circulation after oral administration. Saquinavir is highly metabolized to several inactive metabolites which have not been specifically measured in plasma.

The absolute bioavailability of saquinavir when administered with a high fat meal approximates 4%, which is several fold higher than what was observed during fasting conditions. A significant increase in the bioavailability of orally administered saquinavir in the fed state was evident in all of the studies assessing food effect. A study using gamma scintigraphy revealed that, in the fasted state, the radiolabelled marker emptied 6 times faster compared to the fed state whereas, time taken to reach the colon was similar for both fed and fasted states. Further, it appears that prolonged gastric emptying was associated with higher AUC.

A study to assess the effect of raising gastric pH (by ranitidine) on the pharmacokinetics of saquinavir revealed that the increase in bioavailability was not related to change in the gastric pH. The pH measurements were recorded using a telemetric pH recording device (Flexilog 100).

A study to assess the effect of timing of food intake on the bioavailability of saquinavir indicated that saquinavir should be administered any time within 2 hours after food. Further, the increase in saquinavir bioavailability following food cannot be explained by increase in pH caused by the food intake.

Grapefruit juice has been reported to increase the bioavailability of drugs metabolized by cytochrome p450 3A4 (felodipine, nitrendipine, nifedipine and cyclosporine). The bioavailability of saquinavir increased about 50% to 100% when co-administered with grapefruit juice. However, all the drug and food interaction studies have been conducted in healthy volunteers and thus the effect of these interactions in AIDS patients is unknown.

A high degree of intersubject variability in the pharmacokinetics of saquinavir has been observed, and it is greater after oral than intravenous administration. This supports the assumption that variable absorption and first pass metabolism contribute to the observed variability in saquinavir pharmacokinetics. Saquinavir absorption occurs over a prolonged period of time. Greater than proportional increases in AUC and C_{max} have been observed as saquinavir doses are increased. After multiple dosing, a 2-fold accumulation has been observed.

HIV-Infected Patients

The pharmacokinetics of saquinavir was evaluated in selected patients involved in the clinical studies. All of the patients received oral doses and were instructed to take the drug with food. Patients received doses of 25, 75, 200 and 600 mg tid. Preliminary, unaudited, data from a study in which patients received doses of 600 and 1200 mg q4h (6 times per day) were also included in the NDA. Saquinavir concentrations were found to be about 2-fold higher in patients than in healthy volunteers at equal doses. Attempts were made to establish a pharmacokinetic/pharmacodynamic relationship between saquinavir AUC and surrogate marker responses. It appears that the 600 mg tid dose is active and the lower doses are not. Results from the preliminary analysis of the data from the study using higher saquinavir doses (up to 7200 mg day) suggest that higher exposure leads to greater response. These relationships remain to be further described.

Drug Interactions

Based on in-vitro data, isozyme Cyp 3A4 has been identified as being responsible for saquinavir's metabolism. Ketoconazole is a known potent inhibitor of Cyp 3A4 and rifampin is a known inducer of cytochrome p450 enzymes. Mean saquinavir Cmax, Cave and AUC were about 2.5 to 3 times higher when co-administered with ketoconazole (200 mg qd). Rifampin (600 mg qd) decreased mean saquinavir AUC and Cmax to about 16%

and 21%, respectively, of values obtained when saquinavir was administered alone.

In patients, no significant pharmacokinetic interactions were observed between saquinavir and zdv, ddC or ddI. The coadministration of rifabutin (300 mg/day) with saquinavir (600 mg tid) resulted in a 40% decrease in the AUC of saquinavir.

Special Populations

The pharmacokinetics of saquinavir has not been specifically studied in subjects with renal or hepatic impairment. Saquinavir has not been studied in pediatric patient populations. The numbers of female, minority and elderly study participants were not high enough to make statistical comparisons.

Dissolution

The proposed dissolution method uses
at least dissolved at 45 minutes. **The dissolution method proposed by the Applicant is acceptable, however, the data support a higher Q value than proposed by the Applicant. It is concluded that the data support a specification of Q = in 45 minutes.**

The Applicant felt that a Q of would prevent batches from having to go to Stage 2 dissolution testing and did not want the Q value to be increased. After discussions with the Applicant, it was decided that an interim value of Q would be set and the Applicant would have one year to provide data in support of changing the Q.

Label

The review of the label was ongoing and the final label is on file in the Division.

Phase IV Commitments

The Applicant has commited to the following (relevant to clinical pharmacology / biopharmaceutics): (i) They will investigate INVIRASE in combination with other protease inhibitors, through pharmacokinetic interaction studies and/or surrogate marker studies (ii) They will optimize the dose of INVIRASE through the development of a more bioavailable formulation and (iii) They will conduct a drug interaction study with the improved oral formulation and terfenadine

CONCLUSIONS:

The Applicant has examined the pharmacokinetics of saquinavir in both healthy and HIV-infected subjects. They have described the pharmacokinetics after intravenous infusion and single/multiple oral dosing. In assessing the pharmacokinetics, they have looked at the effect of food, dose proportionality, protein binding, drug interactions and relative bioavailability. The Applicant has also attempted to establish pharmacokinetic pharmacodynamic relationships for saquinavir and surrogate markers. The Applicant established the bioequivalence of the clinical and to be marketed formulations,

and proposed a dissolution methodology and specification. The Applicant has not addressed pediatric dosing concerns.

RECOMMENDATION:

The Human Pharmacokinetics and Bioavailability Section of NDA 20,628 has met the requirements of the Code of Federal Regulations 320 and the clinical pharmacology labeling requirements of the Code of Federal Regulations 201.5E thus, supporting the approval of this NDA.

Advisory Committee - November 7, 1995.
Biopharm Day - October 26, 1995
Participants: Drs. J. Collins, L. Lesko, P. Hepp, M. Mehta, N. Fleischer, J. Lazor, M. Chen, J. Jenkins, C. Sahajwalla.

REVIEW

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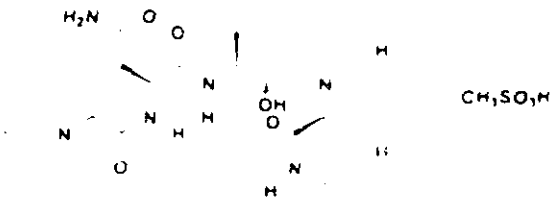
Attachments

I. CHEMISTRY (saquinavir mesylate):

-Chemical Name- cis-N-tert Butyl-decahydro-2[2(R)-hydroxy-4-phenyl-3(S)-[[N-(2-quinolylcarbonyl)-L-asparginyl]amino]butyl]-(4aS,8aS)-isoquinoline-3(S)-carboxyamide methanesulfonate.
-Molecular Formula- $C_{38}H_{50}N_6O_5 \cdot CH_4O_3S$
-Molecular Weight- 766.96 (methanesulfonate) 670.86 (free base)
-Solubility- Maximum aqueous solubility of 0.17 - 0.22 g/100 mL at 25°C in buffer solutions between pH 3-4. In methanol the solubility is 2.0 g/mL and in a 50% ethanol/water mixture the solubility is 1.37 g/100 mL. The pH of a 1% aqueous suspension is ~5.
-octanol/water partition coefficient- log P = 2.12
-pKa- 7.01

-Structure



II. FORMULATION:

INVIRASE hard gelatin capsules will be manufactured in Basle, Switzerland and Nutley, New Jersey. The commercial batch sizes will be
capsules. Three granulating units of comparable size are combined to produce each production scale lot. The commercial formulation differs from the clinical formulation in the addition of talc and a change in capsule color

Ingredient	mg/capsule	Weight %
Saquinavir mesylate (active)		

III. INDICATION AND USAGE (from label):

INVIRASE in combination with nucleoside analogues is indicated for the treatment of advanced HIV infection in selected patients

IV. DOSAGE AND ADMINISTRATION

600 mg (3 x 200 mg capsules) tid. taken within 2 hours after a full meal or substantial snack.

The studies demonstrating the significant increases in saquinavir bioavailability upon administration with food used full meals which contained ~50 grams of fat and ~900

calories. The Applicant did not have a specific definition for a "substantial snack", but indicated that it may be something like a BigMac sandwich (Advisory Committee meeting 11/7/95), which probably more approximates what is intended by a "full meal". The term "substantial snack" is vague and should not be included in the dosing recommendation.

V. PHARMACOKINETICS:

This section contains a summary of the submitted studies. More detailed information regarding the studies is on file in the Division of Pharmaceutical Evaluation III. Saquinavir pharmacokinetic information was obtained from 363 healthy volunteers and 270 HIV-infected patients, in 26 studies.

A. HEALTHY VOLUNTEERS

1. INTRAVENOUS:

Intravenous data are contained in study summaries for the oral studies and food effect studies. In order to avoid repetition, the data have not been included here.

2. ORAL:

a. ^{14}C-Labelled Saquinavir

Single dose pharmacokinetic and excretion balance study after intravenous and oral administration of 14-carbon labelled Ro 31-8959/006 to healthy male volunteers (Protocol WK 14190B)

The objectives were to investigate the routes and rates of elimination, compare urinary recovery (i.v. versus oral, parallel group) and determine the pharmacokinetics of radioactivity and the parent compound. ^{14}C-saquinavir was administered intravenously (N = 4, 10.5 mg free base, infused over 60 minutes) and orally (N = 8, 600 mg free base) to healthy male volunteers and pharmacokinetics were determined.

Results:
Mean plasma levels of total radioactivity and saquinavir are provided in Figures 1 (intravenous) and 2 (oral). The following table summarizes the results:

Parameter	Saquinavir Intravenous Infusion (N = 4)	Saquinavir *Oral (N = 8)*	Radioactivity *Intravenous* Infusion	Radioactivity Oral
AUC* ng.h/mL	131 ± 13.3	146 ± 69.5	200 ± 21.1	1114 ± 288
AUC(0-inf) ng.h/mL	136 ± 16.4		289 ± 32.2	1508 ± 422
Cmax ng/mL	....	39.0 ± 18.8		....
Clearance L/hr	7.8 ± 9.3			....
Half-life (1) hours	0.14 ± 0.03			....
Half-life (2) hours	3.2 ± 1.2	....	13.3 ± 2.53	14.0 ± 2.94
Volume (L)	353 ± 97.8	....	....	....
Total % Recovered	....	....	84.5 ± 6.5	88.8 ± 9.3
% in Urine**	....	....	3.13 ± 0.55	0.98 ± 0.28
% in Feces#	....	....	81.4 ± 6.09	87.8 ± 9.26
Parent % of Radioactivity	66% ± 3.8%	12.7% ± 4.12%	....	....

* AUC(0-12) for i.v. data and AUC(0-12) for oral data
** 0.84 for i.v. and 0.72 for oral
\# 0.268 for i.v. 0.192 for oral

The pharmacokinetic parameters for total radioactivity were computed by the sponsor at the reviewer's request (submission dated 9/28/95). The drug is extensively metabolized and most (>80%) of the radioactivity was *recovered in feces, irrespective* of route of administration. Animal studies suggest that drug is predominantly excreted via bile. Based on total radioactivity recovered in urine the sponsor suggests that about 30% of the drug was systemically absorbed (assuming *similar metabolism for i.v. and oral* drug and absence of gut metabolism). The low absolute bioavailability (about 4%) of saquinavir could be attributed to low absorption and or first pass metabolism. If 30% of the drug is absorbed then it would indicate that low bioavailability must be the result of both low absorption and first pass metabolism. Further evidence of first pass metabolism is presented by comparing the ratio of parent drug to the total radioactivity. Following intravenous administration 66% of the total radioactivity is attributed to the parent whereas, following oral administration only about 13% of the radioactivity is saquinavir. This would indicate that about 80% of the absorbed radioactivity undergoes pre-systemic metabolism (first pass or gut) and thus at least about 20% of the administered drug (radioactivity) is absorbed. Hence the 30% of the administered dose reaching the circulation (metabolized or unchanged) is a reasonable estimate. The sponsor has indicated in their technical summary (vol 146 pg 32) of their NDA that 30% of intact drug crosses the intestinal epithelium, which cannot be substantiated by this study.

b. SINGLE DOSE/FOOD EFFECT/PH EFFECT

Study P-5174 - A study of the tolerability and pharmacokinetics (dose proportionality, relative bioavailability and the 'effect of food') of oral doses of 25, 75, 200, 600, 1200 and 1800 mg of Ro 31-8959 or placebo in healthy human volunteers. (Vol. 177) First time in man

Sixty healthy males between the ages of 19 and 36 participated in this ascending single dose, randomized double blind study which examined the pharmacokinetics of saquinavir at 6 dose levels. Six subjects per group received saquinavir and 4 received placebo. A suspension formulation (60 mg/g in 10% succinylated gelatin) was diluted to 50 mL with water and administered after an overnight fast. The group receiving 600 mg also received 600 mg as a capsule formulation (6 x 100 mg), under fasting and fed conditions (high fat breakfast, description in attachment). Escalation to the next higher dose, was separated by at least 4 days. Samples for measurement of saquinavir were collected prior to dosing and 0.33, 0.67, 1, 1.33, 1.67, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16 and 24 hours after dosing. A 48 hour sample was taken in the 1800 mg dose group and after the last of the three 600 mg doses. Mean (%CV) parameter estimates are in the following table, n = 6 per treatment.

Treatment	C_{max} (ng/mL)	T_{max} (h)	AUC8 (µg*h/mL)	AUC24 (µg*h/mL)
25 mg	0.68 (108.9	0.47	***	***
75 mg	2.15 (79.5)	0.56	3.28 (146.8)	***
200 mg	7.74 (85.3)	0.73	13.69 (88.5)	***
600 mg	9.72 (49.2)	1.39	20.32 (49.7)	40.17 (51.7)
1200 mg	11.35 (83.7	1.00	27.26 (67.4)	59.31 (63.9)
1800 mg	26.46 (58.0	0.50	59.62 (70.1)	97.32 (49.9)
600 mg (cap fasted)	2.99 (51.9)	2.[illegible]	7.74 (55.8)	23.51 (33.4)
600 mg (cap fed)	35.45 (57.9)	[illegible].78	119.1 (38.8)	160.9 (35.0)

*** not calculable

The significant finding from this pilot study was that a profound food effect occurs when saquinavir is administered within 30 minutes after a high fat breakfast. This finding resulted in all subsequent studies being performed under fed conditions. It was also noted that small secondary peaks were evident in some individuals and these peaks occurred around meal time. After administration of the suspension, absorption was relatively rapid and as would be expected T_{max} occurred earlier after suspension than after capsule administration. AUC increased with dose but the increase was less than proportional. The pharmacokinetics of saquinavir exhibited a large degree of variability

Study P-5176 - A single and multiple dose, placebo-controlled, tolerability and pharmacokinetic study of four dosages of Ro 31-8959 in healthy volunteers. (Vol. 178)

Thirty-seven males (1 drop out) between the ages of 19 and 37 participated in this double-

blind, multiple ascending dose study. Saquinavir was administered as capsule formulations (25 and 100 mg). Single doses were given in a randomized fashion after either a light (7.6 g fat, 355 calories) or heavy (53.7 g fat, 943 calories) breakfast. This was followed by multiple dosing (tid) for 7 days. Subjects received placebo (n = 3/group), or saquinavir (n = 6/group) 25 mg, 75 mg, 200 mg or 600 mg tid, with food. A further single dose was given after a heavy breakfast following the multiple dosing period in order to compare saquinavir pharmacokinetics after single and multiple dosing, under fed conditions. Blood samples were collected prior to dosing and 0.33, 0.67, 1, 1.33, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16 and 24 hours after dosing. Single blood samples were also collected prior to the morning doses on days 2 and 6 and prior to the last dose on day 7.

The mean (%CV) parameter estimates after single dose administration are in the following table. Comparison of the individual data revealed that at the 600 mg dose there was an average 2.16 (range 0.69-4.2) fold increase in AUC_{24}, when the heavy breakfast was compared to the light breakfast. Saquinavir pharmacokinetics was not significantly affected by meal content at the lower doses. When compared to the previous pharmacokinetic study, the C_{max} and AUC values after single dose administration of saquinavir (600 mg) were higher in the present study, but were in agreement with those from other studies. Due to the high variability and limited number of subjects, the significance of a cross study comparison cannot be determined.

	'HEAVY' BREAKFAST DAY 1				'LIGHT' BREAKFAST DAY 1			
Treatment	C_{max} (ng/mL)	T_{max} (h)	AUC_8 (µg*h/L)	AUC_{24} (µg*h/L)	C_{max} (ng/mL)	T_{max} (h)	AUC_8 (µg*h/L)	AUC_{24} (µg*h/L)
25 mg	1.25 (105.2)	2.33 (93.5)			0.73 (84.4)	1.16 (68.4)	....	....
75 mg	1.41 (73.5)	1.89 (54.5)	3.24 (78.7)		2.26 (63.5)	1.05 (61.6)	4.51 (84.8)	....
200 mg	11.32 (79.3)	3.72 (41.0)	30.72 (92.5)	35.59 (96.8)	13.58 (98.9)	2.72 (65.6)	31.36 (48.9)	36.09 (52.1)
600 mg	66.08 (95.6)	4.11 (31.5)	190.2 (63.8)	246.2 (58.1)	32.22 (35.3)	2.94 (54.3)	92.42 (32.4)	120.2 (28.6)

The following table contains mean (%CV) parameter estimates for saquinavir after multiple dosing. For the 600 mg dose, there was an average 2.23 (range, 0.6-3.4) fold increase in AUC_8 on Day 8 compared to a single dose. Mean trough concentrations were similar from the 4th dose onward, suggesting that a steady state had been achieved.

	'HEAVY' BREAKFAST DAY 8			
Treatment	C_{max} (ng/mL)	T_{max} (h)	AUC8 (ug*h/L)	AUC24 (ug*h/L)
25 mg	0.47 (64.2)	1.94 (52.6)		
75 mg	2.04 (79.0)	3.13 (74.4)	6.69 (96.1)	
200 mg	11.43 (72.1)	1.86 (37.2)	44.79 (47.0)	63.57 (39.4)
600 mg	90.39 (48.8	3.29 (33.4)	359.0 (46.0)	492.9 (44.7)

Study P-8016 - A single randomized, cross-over study of the absolute and relative bioavailability of Ro 31-8959 in healthy volunteers. (Vol. 199)

The absolute and relative bioavailability of capsule (formulation 014, 200 mg capsules) and suspension (formulation 012, 60 mg/g) formulations of saquinavir were determined in 9 healthy male volunteers. The disposition of saquinavir after an one hour intravenous infusion (formulation 007) was also assessed. The three treatments (each separated by 7 days) were administered in a randomized fashion, after a standardized breakfast. The oral dose was 600 mg and the iv dose was 12 mg. Blood samples were collected over a 48 hour period. One subject had a respiratory illness and did not complete the study. The mean (% CV) parameter estimates are in the following tables:

Parameter	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	AUC8 (ug*h/L)	AUC_∞ (ug*h/L)	CL/F (L/h)	F (%)
Capsule (n=8)	65.8 (71.6)	3.8 (113.9)	10.2 (32.5)	173.6 (64.6)	222.0 (63.4)	4215 (84)	3.8 (72.8)
Suspension (n=9)	65.3 (53.9)	2.9 (37.3)	4.8 (60.1)	189.1 (59.7)	237.8 (53.9)	3229 (52)	4.0 (51.2)

Parameter	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	AUC8 (ug*h/L)	AUC_∞ (ug*h/L)	CL (L/h)	Vd
IV infusion (n=9)	87.4 (14.6)	1.0 (11.4)	6.7 (31.9)	89.1 (13.7	106.0 (14.3)	102.2 (13.5)	1447.0 (38.4)

The absolute bioavailability of saquinavir was approximately 4% (range, 0.85-9%) after the administration of capsules or suspension. The mechanisms responsible for the low bioavailability have not be determined but are possibly related to poor absorption and/or high first pass elimination. The mean pharmacokinetic parameters were similar for the two oral formulations, however, evaluation of the individual data reveals that there was a high degree of variability between subjects. Some of the variability is likely due to variations in individual metabolic activity, as the variability after iv administration was much less. The half-life tended to be longer after oral administration which may be the result of prolonged absorption from the GI tract. The following figure contains mean concentration versus time profiles for the three formulations over 48 hours and for the first 8 hours.

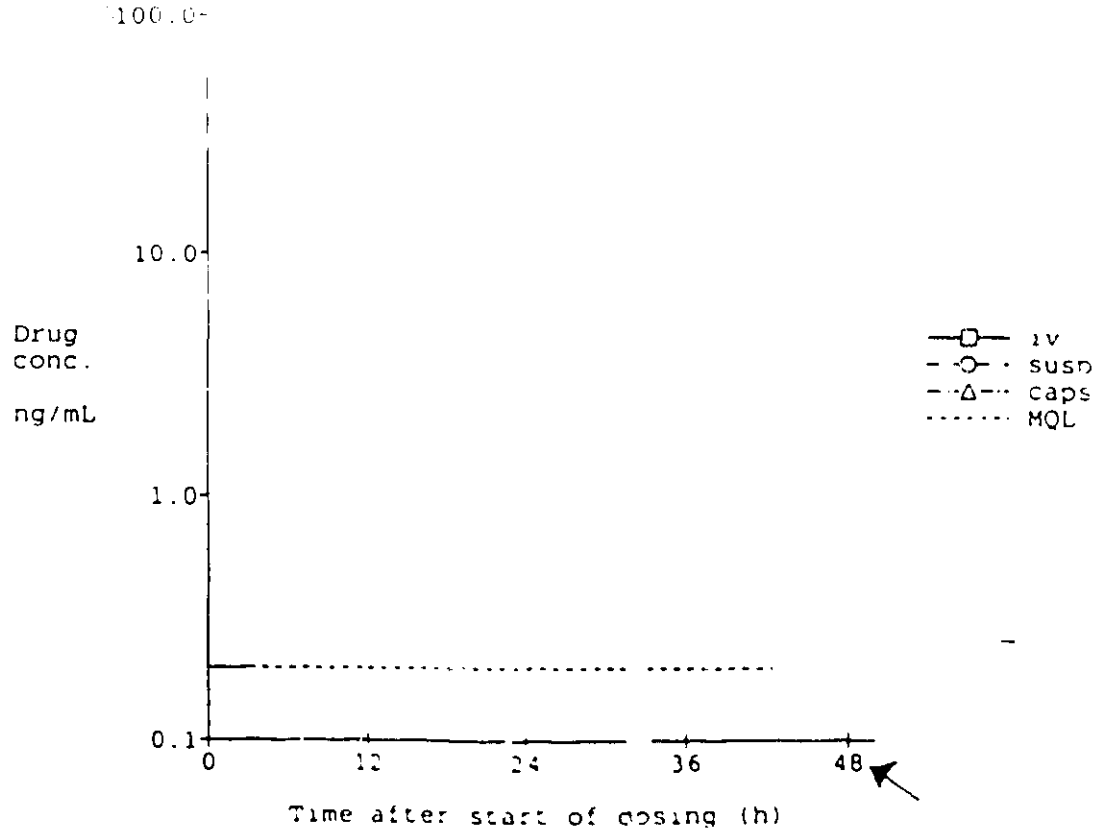


Figure 2A Mean plasma concentrations of Ro 31-8959 after an infusion of 12 mg in 1 h (iv) an oral 600 mg suspension (susp) and an oral dose of 600 mg in capsules (caps)

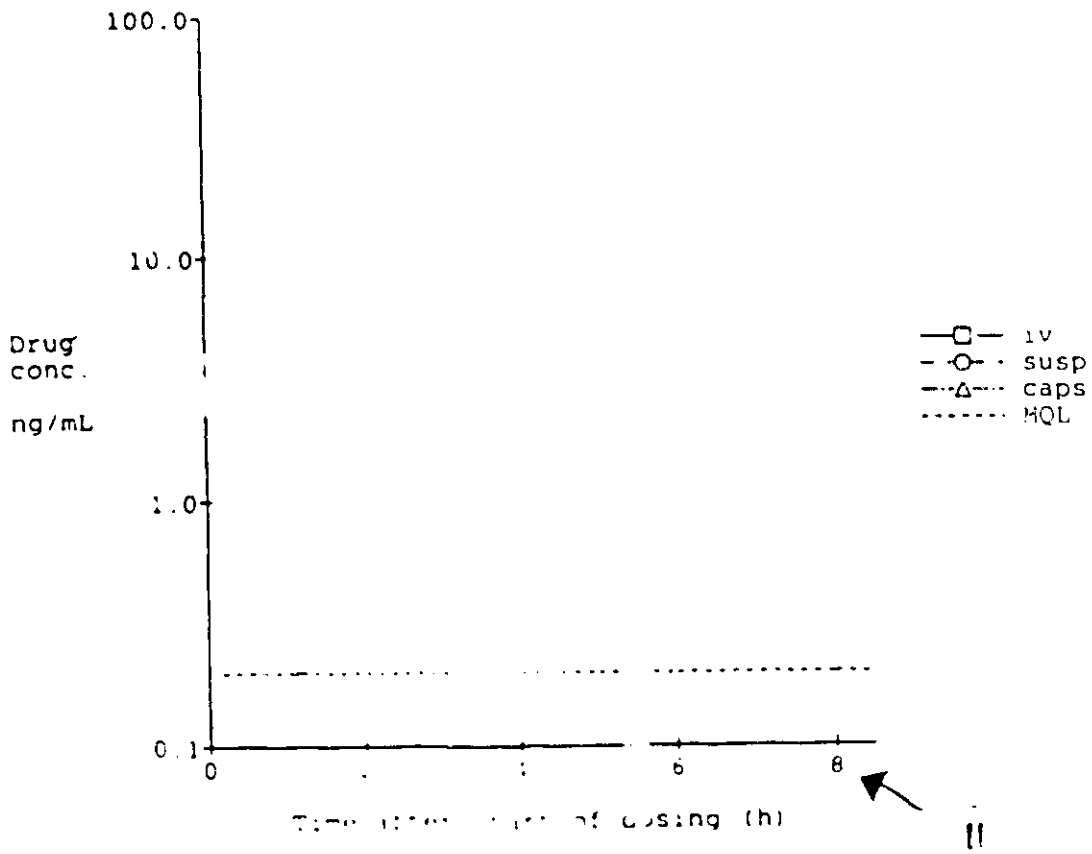


Figure 2B Mean plasma concentrations of Ro 31-8959 after an infusion of 12 mg in 1 h (iv) an oral 600 mg suspension (susp) and an oral dose of 600 mg in capsules (caps)

After iv administration, concentrations fell rapidly with the majority of the total AUC being accounted for in the first 8 hours. There were some irregularities in individual profiles such that concentrations decreased at some times during the infusion and C_{max} was reached at 40 minutes for one subject. Overall, these irregularities are not felt to have had a significant impact on the interpretation of the study data. The systemic CL and V_d were large. Systemic CL exceeded hepatic blood flow, indicative of extra-hepatic metabolism. The iv data were also analyzed using compartmental methods which employed a 3-compartment model. The CL was comparable to that determined after the noncompartmental method, averaging (%CV) 98.8 (14.5) L/h. Volume of the central compartment averaged 12.3 (31) L, consistent with distribution to extracellular space (15 L). The Vss averaged 703 (39) L, suggesting partitioning into or binding to tissues. The half-life of the terminal phase was about 12 hours and the mean residence time was 7 hours. The half-life determined from the compartmental analysis was closer to that observed after oral administration. The half-lives may be reflective of slow release from tissue binding sites. Sixty-two percent of the variation in CL could be accounted for using the equation from the linear regression relationship between CL and bodyweight (subject 7 was not included in the analysis due to anomalous results). There was no apparent relationship between Vss and bodyweight. Visual examination of actual versus modelled data showed that the model was able to describe the study data (see following figure).

Study WK14939 - An open label bioequivalence study of the proposed market formulation (/622) of saquinavir (Ro 31-8959) relative to the clinical trial formulation (/014) in healthy male volunteers. (Vol. 204) - Pivotal Bioequivalence Study

Twenty-four healthy males participated in this randomized, 3-way crossover study which assessed the bioequivalence of clinical and to be marketed formulations of saquinavir. The granulates for the two formulations differ in that talc is added to formulation /622. Subjects received two 200 mg capsules of the study formulations along with one 200 mg capsule of the deuterated formulation (/079, batch size 1,000 capsules) to make total single saquinavir doses of 600 mg. Drug was administered after a standardized breakfast and study days were separated by at least 6 days. The breakfast consisted of 1 bowl of corn flakes with 100 mL of whole milk, 2 rashers of lean bacon, 2 fried eggs, 2 slices of toast with butter, 100 mL of fresh orange juice and 150 mL of decaffeinated tea or coffee (breakdown of meal content was not reported, but it appears to be high fat). Treatments were as follows: A) saquinavir, /014 batch size capsules. B) saquinavir, /622 market formulation, batch size capsules and C) saquinavir, /014 capsules. Samples were collected for 12 hours post dosing. The mean (%CV) data from the study are in the following table. The intended market formulation (/622) was bioequivalent to the formulation used in clinical trials (/014). The reserve formulation (/014 Welwyn) did not meet the requirement for bioequivalence because the upper limit of the confidence interval for C_{max} was 127% which is higher than the acceptable 125%. This formulation has been used only for stability assessments in Nutley (per Applicant 9/29/95).

*** The Welwyn process involved the manufacture of saquinavir on production scale equipment using the clinical trial formulation which did not contain talc

Plasma concentrations of Ro 31-8959 after an i.v. infusion of 12 mg (dashed line) compared with modelled data (solid line)

Plasma concentrations of Ro 31-8959 after an i.v. infusion of 12 mg (dashed line) compared with modelled data (solid line)

Parameter mean (%CV)	Treatment A /014 phase II III formulation	Treatment B /622 market formulation	Treatment C 014 Norwalk process	90% CI B relative to A	90% CI C relative to A
AUC_{12} (µg*h/L)	150.8 (81.9)	151.5 (72.9)	147.7 (50.1)	**********	**********
C_{max} (ng/mL)	52.8 (63.9)	51.2 (76.6)	49.6 (49.5)	**********	**********
T_{max} (h)*	4.5	4.5	4.5	**********	**********
T_{lag} (h)*	0.5	0.5	0.5	**********	**********
AUC_{12} µg*h/L	72.9 (75.1)	71.7 (74.6)	69.0 (52.5)	**********	**********
C_{max} (µg/mL)	26.0 (52.9)	26.1 (73.7)	23.8 (56.0)	**********	**********
T_{max} (h)	4.5	4.5	4.5	**********	**********
T_{lag} (h)	0.5	1	0.5	**********	**********
RAUC@ -Arithmetic -Geometric	2.1 (22.9) 2.0	2.2 (29.2) 2.2	2.2 (18.5) 2.2	99 116 mean 107%	100 117 mean 108%
RC_{max}@ -Arithmetic -Geometric	2.0 (28.2) 1.9	2.0 (25.9) 2.0	2.2 (28.8) 2.1	88 118 mean 102%	95 127 mean 110%

* median

@ relative AUC and C_{max} are the ratios of the respective values from the unlabelled capsule dose to the values from the concurrently administered labelled dose

The statistical analysis performed by the Applicant used a 3-way analysis of covariance with factors for treatment, subject and period (or date, instead of period). A consultation with the Division of Biometrics (Donald Schuirmann) regarding the analysis was sought on 11/3/95. It was concluded that the methods used by the Applicant were acceptable. When data from the Applicant's ANOVA tables were used to approximate standard error and estimate confidence intervals using the 2 one sided test, results similar (within 1 to 3 percent) to those reported by the Applicant were obtained. It is also noted that AUC_{12} and not AUC_∞ was used in the bioequivalence assessment. One of the difficulties in estimating AUC_∞ for saquinavir is that absorption is prolonged, such that an accurate determination of the elimination rate constant (needed for extrapolation) cannot be made. A comparison of the percent of the total AUC accounted for at 8, 12 or 24 hours, in other studies, revealed that the majority of the AUC was accounted for by 8 hours. For this study, it is felt that AUC from 12 hours to infinity probably contributes minimally to the overall AUC, since concentrations were low at 12 hours. Taking this into account along with the impact of assay variability at lower concentrations, it is concluded that the use of AUC_{12} was acceptable.

The Applicant performed a number of bioequivalence studies as they attempted to identify suitable formulations of saquinavir. The reference formulation for these studies was 014 (Clinical formulation). Pharmacokinetic data for formulation 014 have been extracted from the bioequivalence studies which will not undergo review for this NDA approval and are summarized in the remainder of this section.

Study WK14938- An open label study to compare the bioavailability of different formulations of saquinavir relative to the clinical trial formulation (/014) in healthy volunteers. (Vol. 170)

Mean (% CV) data from 23 of 24 healthy volunteers (11 ♂, 12 ♀) who received 600 mg of saquinavir (400 mg of formulation 014 and 200 mg of deuterated formulation) after a standardized breakfast are in the table below. Deuterated saquinavir was administered as an *in vivo internal standard to reduce the large intra- and inter-individual variability*. Blood samples were collected up to 12 hours post dose. Urinary concentrations of 6 β-hydroxycortisol and cortisol were measured to assess cytochrome P450 activity in order to possibly explain the source of *the variability in saquinavir pharmacokinetics*. The data from this assessment demonstrated a decrease in the ratio of 6 β-hydroxycortisol/cortisol in the treatment groups compared to ratios measured at screening, while the ratios were not significantly different among the treatment groups. These results are consistent with a reduction in ratio upon treatment with saquinavir. There was no correlation between the ratios and pooled saquinavir AUC, AUC assessed by treatment or sex, indicating that the ratio cannot be used to explain the variability in saquinavir pharmacokinetics. Linear regression of the plot of labelled and unlabelled AUC (pooled from all treatments) resulted in an r^2 of 0.81, demonstrating good correlation between AUC values for the different forms of drug. ANOVA analysis of the rel_AUC and rel_Cmax determined the within subject %CV ($\sqrt{\exp(\sigma^2)-1}$) to be 19.1 and 28.8, respectively

Parameter	AUC12 (µg*h/L)	Cmax (ng/mL)	Tmax (h), median	T½ (h), median	rel. AUC*	rel. Cmax*
Unlabelled	147.4 (54.0)	48.2 (57.1)	4.5	1.0	2.7 (21.3)	2.5 (22.1)
Labelled	59.3 (59.7)	20.7 (60.4)	4.5	1.5	.....	.....

* relative AUC and Cmax are the ratios of the respective values from the unlabelled capsule dose to the values from the concurrently administered labelled dose

Other studies using formulation 014

The following table contains mean (%CV) pharmacokinetic parameters for formulation /014 from bioequivalence studies performed under fed conditions during drug development:

Study Location	Dose	Cmax (ng/mL)	Tmax (h)	AUC12 (µg*h/L)
WK14338 Vol. 174	600 mg	108.1 (47.1)	4.0 (31)	291.7 (65)
WK14508 Vol. 175	600 mg	61.4 (69)	3.7 (28)	171.1 (58)
WK14538C Vol. 197	400 mg	31.26 (60.8)	4.5 (3.3)	96.5 (67.9)
WK14956A Vol. 205	400 mg	49.8 (52.6)	4.5 (range)	147.2 (57.6)

c. ADDITIONAL FOOD EFFECT/ PH EFFECT

An open label study of the effect of grapefruit juice on the pharmacokinetics of saquinavir (Ro 31-8959) in healthy volunteers.

It has been reported that plasma concentrations of felodipine, nitrendipine, nifedipine and cyclosporine increased when these drugs (metabolized by cytochrome p450 3A4) were administered with grapefruit juice. Grapefruit juice contains flavonoids which are believed (in-vitro data) to be inhibitors of CYP 3A4. Since saquinavir is metabolized via CYP 3A4, the present study was to compare the bioavailability of saquinavir from three single oral doses of 600 mg when administered in presence or absence of grapefruit juice. This was a 3-way, randomized, cross-over (6 days wash out period) study conducted in 12 healthy subjects (6M and 6F). Saquinavir plasma concentrations were determined between 0 to 12 hours following each treatment.

TREATMENT A: 600 mg saquinavir administered in absence of grapefruit juice.
TREATMENT B: 600 mg saquinavir adrninistered in the presence of double strength grapefruit juice (*Delmonte brand, selected since reported studies for other drugs* used this brand).
TREATMENT C: 600 mg saquinavir administered in the presence of single strength grapefruit *juice (Safeway brand, because conveniently available throughout the* country).

(single strength = reconstituted according to label instructions; double strength = reconstituted using half the required volume of water)

Treatments B and C were administered with 150 mL of grapefruit juice with additional 150 mL at one hour after administration of the drug

Results:
Mean plasma concentrations of saquinavir after single dose (three treatments) are provided in Figure 5. The following table summarizes the results

Total N = 12. Males = 6 and Females = 6 Mean (%CV)	Cmax ng/mL			$AUC_{0-\infty}$ ng h mL			Tmax* (h)		
	Male	Female	Overall	Male	Female	Overall	Males	Female	Overall
Treatment A	76.5 (65.9)	40.88 (43.5)	58.7 (57.4)	227.6 (61.4)	148.7 (53.1)	183.2 (61.9)	4.75	3.25	4.0
Treatment B	120.6 (49.3)	107.1 (45.6)	113.9 (46.0)	374.3 (52.1)	389.1 (41.5)	374.4 (45)	4.5	4.0	4.25
Treatment C	92.1 (50.3)	78.9 (49.5)	85.48 (48.5)	254.0 (64.1)	232.5 (44.8)	238.1 (45.5)	3.75	4.5	4.5

* Median values for Tmax

volunteers. Plasma samples measured during the infusion and up to 33 hours following start of infusion.

TREATMENT A: 6 mg (2 mg/h for 3 hours)
TREATMENT B: 36 mg (12 mg/h for 3 hours)
TREATMENT C: 72 mg (24 mg/h for 3 hours)
TREATMENT D: 72 mg (12 mg/h for 6 hours)

All subjects fasted overnight and those receiving treatments A, B and C were fed a standardized breakfast (1 bowl cornflakes with 150 mL whole milk, 2 rashers lean bacon, 2 fried eggs, 2 slices toast with butter and 200 mL decaffeinated tea or coffee) 30 minutes before the infusion. Subjects in treatment D consumed their breakfast 3 hours after the start of the infusion.

In an absolute bioavailability study, the clearance of saquinavir was found to be greater than hepatic blood flow, suggesting extra-hepatic clearance. To investigate this finding, hepatic blood flow was measured by determining indocyanine green (ICG) clearance. All groups received 40 mg of ICG intravenously, immediately following saquinavir infusion.

Local intolerance (erythema and swelling) to infusion resulted in premature termination of the study. This intolerance was attributed to presence of glycofurol in the formulation and/or intolerance to saquinavir.

Results:
Mean plasma concentrations of saquinavir after single dose (four i.v. treatments) are provided in Figure 7. The pharmacokinetic parameters obtained are summarized in the following table. The steady state concentration (Css) was defined as Cmax within the infusion period (0 to 3.hours) and for treatment D, Css under fed state was defined as concentration at 5 hours following start of the infusion.

Insert table from pg 31, volume 201 of the study report.

Table 7: Mean Pharmacokinetic Parameters for Ro 31-8959 and Total Venlafaxine

Treatment A: 9 mg (2 mg/8)

Parameter / Treatment	Css* ng/mL	tmax h	AUCt ug.h/L	CLt L/h	CL/(CG) L/h
Mean	30.82	2.125	77.25	86.80	5...7
%CV	24.91	40.18	38.40	37.34	17.01
n	4	4	4	4	3

Treatment B: 36 mg (12 mg/h)

Parameter / Treatment	Css* ng/mL	tmax h	CL0-3 L/h	AUCt ug.h/L	CLt L/h	CL/(CG) L/h
Mean	174.8	2.200	74.61	558.6	66.24	59.07
%CV	14.57	34.47	16.25	16.84	20.26	22.04
n	5	5	5	5	5	3

Treatment C: 72 mg (24 mg/h)

Parameter / Treatment	Css* ng/mL	tmax h	AUCt ug.h/L	CLt L/h	CL/(CG) L/h
Mean	308.0	2.300	1057	66.54	41.85
%CV	6.139	24.79	7.232	7.228	35.62
n	5	5	5	5	4

Treatment D: 72 mg (72mg/6h)

Parameter / Treatment	Css0-3* ng/mL	Css3-6** ng/mL	tmax h	CL0-3* L/h	CL3-∞* L/h	AUCt ug.h/L	CLt L/h	CL/(CG) L/h
Mean	250.7	153.8	3.111	57.30	83.07	1254	57.32	55.04
%CV	26.76	29.81	>0.22	26.97	27.02	19.70	19.67	24.62
n	6	5	6	6	6	6	6	4

* C_{max} used as best estimate of C_{ss}
** C_{3h} used as best estimate of C_{ss3-6}
* Fasted
** Fed

Individual steady state plasma concentrations (Css) for treatments A, B and C (administered with food) appear to be dose proportional (Figure 8, r^2 = 0.97 and intercept not significantly different than zero) suggesting constant systemic clearance. The Css_{0-3} for treatment D (251 ng/mL) was higher than Css for treatment B (175 ng/mL), which decreased to 154 ng/mL (Css_{0-6}) after subjects were fed during the infusion. The clearance during fasted state was about 24% (treatment B versus D) and 31% (within treatment D) lower compared to fed state. This increased clearance in fed state may be due to transient increase in hepatic blood flow after meals.

The mean plasma clearance of ICG ranged between 42 to 59 L/h. With an average hematocrit of 0.4, these plasma clearances correspond to blood clearance of about 85 L/h. In contrast to the previous study, the saquinavir clearance in the present study was less than the blood clearance of ICG (less than hepatic blood flow). The results from the present study lend support to the assumption that extra hepatic metabolism may not be involved in the elimination of saquinavir.

In summary, when saquinavir is infused in the fed state, its pharmacokinetics appear to be linear. The clearance of saquinavir during the fasted state was lower compared to the fed state. The saquinavir clearance (ICG data) possibly lacks extra hepatic elimination and is cleared via hepatic route.

Title: An open label study to investigate the gastrointestinal transit and absorption of saquinavir using gamma scintigraphy in healthy volunteers (14341)

This was a randomized crossover study in 8 (4M, 4F) healthy volunteers. Subjects were administered a single dose of 600 mg saquinavir (3*200 mg radiolabelled with 1MBq of 153Sm; i.e. 0.33 MBq per capsule) under fed and fasted conditions, separated by 6 days. Plasma concentrations of saquinavir were determined and scintigraphic images using gamma camera were recorded up to 12 hours following each treatment (Fed and Fasted).

TREATMENT A:	600 mg saquinavir administered 5 minutes after standardized breakfast (1 bowl cornflakes with 100 mL whole milk, 2 rashers lean bacon, 2 fried eggs, 2 slices toast with butter and 150 mL decaffeinated tea or coffee)
TREATMENT B:	600 mg saquinavir administered under fasting condition

Results:
Mean plasma concentrations of saquinavir after single dose (Fed and Fasting) are provided in Figure The following two tables summarize the results:

Mean Pharmacokinetic Parameters

	Treatment A (Fed)				Treatment B (Fasting)		
	AUC_{0-12} ng.h/mL N = 8	Cmax ng/mL N = 8	Tmax h N = 8	*Tlag h N = 8	AUC_{∞} ng.h/mL N = 4	Cmax ng/mL N = 4	Tmax h N = 4
Mean	197.5	61.69	4.75	1.25	27.22	11.52	1.50
%CV	58	69.4	17.8	42.8	63.3	46.5	27.2

* Tlag defined as time prior to first observed concentration

Mean Gastrointestinal Transit Parameters

	Treatment B (Fasted)			Treatment A (Fed)		
	T50% GE(h)	T50% SIT(h)	T50% CA(h)	T50% GE(h)	T50% SIT(h)	T50% CA(h)
Mean	0.3	3.6	4.0	1.8	2.7	4.4
%CV	100	33	35	50	44	16

T50% GE(h) Time taken for 50% to empty from stomach
T50% SIT(h) Small intestine transit time
T50% CA (h) Time taken for 50% to arrive in colon

Administering saquinavir in presence of food increased its mean AUC by seven fold. In the fasted state the radiolabelled marker emptied 6 times faster (mean data) compared to fed state. However, time taken for the marker to reach the colon was similar for both fed and fasted states.

In the fasted state, measurable saquinavir concentrations were only available in four of the subjects; therefore, no attempt was made to correlate saquinavir concentration to GI transit parameters. In fed state, there appears to be a correlation (r = 0.786, Figure 10) between the rates of gastric emptying and lag time. Further, it appears that prolonged gastric emptying was associated with higher AUC. Second peak after lunch indicates a possibility of absorption from the colon. However, no firm conclusions about association and/or correlations of GI parameters and saquinavir concentrations can be derived from this study.

Title: A study of the effect of raising of gastric pH on the pharmacokinetics of Ro 31-8959 in healthy volunteers (14159A)

This study was conducted to determine if the increase in bioavailability of saquinavir in fed state is due to a temporary increase in gastric pH. Saquinavir was administered (with either food or ranitidine or both) in a randomized three way crossover design to 12 healthy males.

TREATMENT A: 600 mg saquinavir after ranitidine, without food. (Note: Two 150 mg ranitidine doses were administered orally, one 12 hours pre-dose and a second dose given 1 hour predose.)
TREATMENT B: 600 mg saquinavir after ranitidine, with food
TREATMENT C: 600 mg saquinavir without ranitidine, with food.

A telemetric pH recording device [redacted] was swallowed about half an hour before

the first ranitidine dose. The pH measurements were recorded every 6 seconds between one hour pre saquinavir dose to 8 hours post dose. Blood samples for saquinavir determinations were collected up to 24 hours.

Results:
Mean plasma concentrations of saquinavir after single dose (three treatments) are provided in Figure 11. The table on the following page summarizes the results.

In the following table, pHmax is maximum pH recorded from dosing up to Tmax for each subject, pH5-10 is the pH value obtained for the period 5 to 10 minutes following dosing and Tmax(pH) is time to pHmax.

Table 6 Summary of Pharmacokinetic and Gastric pH Parameters

Treatment A

Subject	pHmax	Tmax(pH) (min)	pH≥ 10	Cmax (ng/mL)	Tmax (min)	AUC (ug h/L)

Treatment B

Subject	pHmax	Tmax(pH) (min)	pH≥ 10	Cmax (ng/mL)	Tmax (min)	AUC (ug h/L)

Treatment C

Subject	pHmax	Tmax(pH) (min)	pH≥ 10	Cmax (ng/mL)	Tmax (min)	AUC (ug h/L)

The relative bioavailability of saquinavir with ranitidine under fasting state versus saquinavir with food alone (treatment C) was 15.9% (C.I. 10.0 - 25.2%). Whereas, the relative bioavailability of saquinavir with ranitidine under fed state w.r.t saquinavir with food alone was 167% (C.I. 106 - 265%). Since C.I. did not include 100% suggests that mean AUC values were significantly different (A < C < B). It should be noted that saquinavir AUC following ranitidine and food increased significantly in all except two patients.

Mean gastric pH between 1 hour predose and 8 hours following dosing are presented in figures 12, 13 and 14. Ranitidine produced greater increases in pH as compared to food alone. pH is also provided in the previous table. Saquinavir AUCt was not correlated with either pH(5-10) or pHmax (Figures 15 and 16).

Saquinavir concentrations following ranitidine in fed state were higher than when the drug was administered with food alone. It appears that this increase was not related to change in the gastric pH, because when the drug was administered with ranitidine in the fasting state the bioavailability was not high.

Saquinavir is best dissolved between pH of 3 to 4 (discussion with reviewing Chemist) and reducing or increasing the pH reduces its solubility. Based on the solubility profile of saquinavir at different pH, one would not expect that increasing pH above 4 would increase its solubility.

***Title*: A pharmacokinetic study of the effect of timing of food intake on the bioavailability of Ro 31-8959 in healthy volunteers (14109A)**

Saquinavir was administered in a randomized three way crossover design to 12 healthy males. The objectives were to asses effect of timing of food intake on the bioavailability and relate any bioavailability differences to gastric pH

TREATMENT A: 600 mg saquinavir 0.5 hours before breakfast (1 bowl cornflakes with 150 mL whole milk, 2 rashers lean bacon, 2 fried eggs, 1/2 slice toast with butter and 200 mL decaffeinated tea or coffee)
TREATMENT B 600 mg saquinavir 2 hours after breakfast
TREATMENT C 600 mg saquinavir 5 minutes after breakfast

All subjects received a standardized lunch 5 hours after and dinner 10 hours after the breakfast.

A telemetric pH recording device was swallowed about an hour before the dose. The pH measurements were recorded every 6 seconds up to 8 hours post dose. Blood samples for saquinavir determinations were collected up to 24 hours.

Results:

Mean plasma concentrations of saquinavir after single dose (three treatments) are provided in Figure 16. The table on the following page summarizes the results. The following table, also contains pHmax, pH5-10 and Tmax(pH) as defined in the earlier study.

The relative bioavailability of saquinavir from Treatment A versus Treatment C was 30% (C.I. 19.3 - 38.5%) based on AUC and 33.6% (C.I. 22.2 - 51.0) based on Cmax. Similarly, the relative bioavailability of saquinavir from treatment B w.r.t treatment C was 110% (C.I. 73.3 - 146.5%) and 123% (C.I. - 80.5 - 185.6%) based on AUC and Cmax respectively. Treatment B and C appear to have similar relative bioavailability.

Mean gastric pH between 1 hour pre dose to 8 hours following the dose are presented in figures 17, 18 and 19. The standard breakfast produced an increase in gastric pH (pH is provided in the previous table). Saquinavir Cmax or AUCt was not correlated with either pH or pH5-10 (Figures 20 and 21).

In summary, it appears that gastric pH did not effect the bioavailability. However, based on the solubility profile of saquinavir at different pH, one would not expect that increase in pH would increase its solubility. Thus the increase in saquinavir bioavailability following food cannot be explained by increase in pH caused by food intake. This study also suggests that saquinavir should be administered within 2 hours after food.

Table 7 Summary of pH parameters

Treatment A

Subject	pHmax	Tmax(pH) (min)	pH>10	Cmax (ng/mL)	Tmax (min)	AUC (ug h/L)

Treatment B

Subject	pHmax	Tmax(pH) (min)	pH>10	Cmax (ng/mL)	Tmax (min)	AUC (ug h/L)

Treatment C

Subject	pHmax	Tmax(pH) (min)	pH>10	Cmax (ng/mL)	Tmax (min)	AUC (ug h/L)

d. MULTIPLE DOSE

Study WK14339B - A pharmacokinetic study of Ro 31-8959 given in three different regimens of multiple oral doses to healthy volunteers. - (Vol. 179)

The Applicant states that the systemic availability of saquinavir may be influenced by a combination of factors such as dosage, drug solubility in gastric contents, gastric emptying rate, drug concentrations in the hepatic portal vein and the activity of the metabolizing enzymes in the liver. This randomized, crossover study was done to determine if changing the frequency of saquinavir dosing would optimize bioavailability. Twelve healthy males received multiple doses of saquinavir for 5 days in regimens of 900 mg bid (practical alternative to tid dosing), 1800 mg qd (intended to test saturability of first-pass metabolism) and 600 mg tid (reference, given at interval of 6, 6 and 12 hours), with each period separated by 2 days. Saquinavir was given after consumption of a meal. Subjects received the first dose of each day at the study site, after eating the standardized breakfast. Pharmacokinetic profiles (q 1 hour sampling) were obtained over a 24 hour period which began after the first dose on the final day of each period. One subject withdrew in the final day of the 1800 mg qd regimen, due to gastritis. A plot of the mean concentration vs. time profiles after the three regimens follows. The mean (%CV) parameter estimates over 24 hours and over individual dosing intervals are summarized in the tables below. Cmax and Tmax were determined from the first dose on the day of the pharmacokinetic profiles.

Regimen	Cmax (ng/mL)	AUC24 (ug*h/L)	Tmax (h), median	Tlag (h), median
A = 900 mg bid (n = 12)	245.1 (50.0)	1198 (39.6	3.5	0
B = 1800 mg qd (n = 11)	288.0 (39.9)	1216 (37.9	4.0	0
C = 600 mg tid (n = 12)	120.1 (43.7	924.6 (34.7	8.0	0

Regimen	Cmax (ng/mL)	Tmax (h), median	AUC6 (ug*h/L)	Cmax (ng/mL)	Tmax (h), median	AUC12 (ug*h/L)
A (n = 12)	245.1 (50.0)	3.5	746.6 (41.8)	9.1 (44.4	5.0	451.9 (36.3)

Regimen	Cmax (ng/mL)	Tmax (h), median	AUC8 (ug*h/L)
B (n = 11)	288.0 (39.9)	4.0	1216 (37.9)

Regimen	Cmax (ng/mL)	Tmax (h), median	AUC8 (ug*h/L)	Cmax (ng/mL)	Tmax (h), median	AUC6 (ug*h/L)	Cmax (ng/mL)	Tmax (h), median	AUC12 (6)* (ug*h/L)
C (n = 12)	98.1 (64.2)	5.5	577.8 (60.7	84.2 (4.2)	6.5	271.9 (38.9)	57.11 (49.8)	5.0	226.7 (44.7)

*6 hour AUC was calculated to allow for comparison with the last 6 dosing intervals

Regimens A & B had similar saquinavir exposure, which was significantly higher than that from Regimen C. However, the concentration vs time profiles indicate that Regimen C provided lower fluctuations and yielded sustained plasma concentrations. For all subjects on Regimen A, C_{max} was lower after the second dose compared to the first and 10 of the 12 had lower AUCs after the second dose. The AUC ranged from 282-1215 µg*h/L after the first 900 mg dose and from 179-786 µg*h/L after the second dose. AUCs were not significantly different for the dosing intervals during Regimen C, however, the C_{max} was lower in the third interval compared to the first two. It was concluded, by the Applicant, that the results from this study were not compelling enough to warrant changing the regimen from a tid to either bid or qd. It is agreed that a change in regimen was not warranted.

PAGE
PURGED

e. DRUG INTERACTIONS

Pharmacokinetic interaction between Ro 31-8959 and ketoconazole in healthy volunteers (protocol No. WK 14435)

Based on in-vitro data, isozyme Cyp 3A4 has been identified as being responsible for saquinavir's metabolism. Ketoconazole is a known potent inhibitor of Cyp 3A4.

The pharmacokinetic interaction of saquinavir and ketoconazole at steady state was studied in this 3-way, randomized, cross-over study conducted in 12 healthy males.

TREATMENT A: 600 mg saquinavir tid (8:00, 14:00 and 20:00 hours) for 5 days, plus a single dose on day 6
TREATMENT B: 600 mg saquinavir tid for 5 days, and a single dose on day 6 plus 200 mg ketoconazole QD for 6 days
TREATMENT C: 200 mg ketoconazole QD for 6 days

Treatments were separated by 1 day i.e., next treatment commenced 24 hours after the final dose of the previous regimen and all the treatments were taken within 10 minutes after food.

Results: Mean steady state plasma levels of saquinavir when administered with and without ketoconazole, are provided in Figure 3. The following table summarizes the results:

Mean %CV (Range)	Tmax (h)	Cmax ng/mL	*Cave ng/mL	AUC_{τ} ng h/mL	$AUC_{0-\infty}$ ng h/mL	$t_{1/2}$ h	Cmax ratio	**AUC ratio	F_{rel} Geo Mean
Saquinavir alone	3.25 47	123.3 47	60.12 42	361 42	677 50	10.75 12	.....	....	....
Saquinavir (with Ketoconazole)	3.58 45	336.6 68	175.02 68	1050 68	1701 58	8.69 26	2.96 56 (0.77-5.71)	2.59 46 (1.06-4.33)	2.30
Ketoconazole alone	3.92 32	3986 22	2161 24	12970 24	24090 17	2.5 25	....	....	....
Ketoconazole (with Saquinavir)	3.33 37	3361 37	2086 40	12510 4	22710 14	2.63 25	....	....	....

* Cave calculated as AUC_{τ} / Tau
** AUC ratio of AUCinf (AUC ratio of AUC_{τ} was 3.02 : 1.67)

Mean saquinavir Cmax, Cave and AUC were about 2.5 to 3 times higher when the drug was co-administered with ketoconazole. Whereas, saquinavir did not have any marked effect (except that ketoconazole Cmax was significantly higher) on the pharmacokinetics of ketoconazole. It should also be noted that 3 of the 12 subjects did not have a significant change in saquinavir pharmacokinetic parameters, when it was co-administered with ketoconazole. Further, increase in concentration was not associated with initial (saquinavir

ketoconazole. Further, increase in concentration was not associated with initial (saquinavir alone) low concentrations of saquinavir.

Increase in Cmax with no change in Tmax suggests that the rate of appearance of saquinavir was not affected. Increase in Cmax, AUC and relative availability could be explained by inhibition of the metabolizing enzyme. In absence of data from the second and third dose saquinavir, it could only be hypothesized that inhibition of the metabolizing enzyme would occur even when ketoconazole concentrations are low resulting in the second and third saquinavir doses also being affected.

It should be noted that 200 mg ketoconazole and 600 mg saquinavir were administered together. To obtain the maximum inhibition that ketoconazole can produce, ketoconazole has been reported to be administered two hours before administering the drug being evaluated. Further, clinically ketoconazole is also given as 400 mg qd. Thus, the ketoconazole interaction reported from this study should be considered as the minimum increase that could be expected and in reality the increase in exposure of saquinavir could be higher.

Based on available safety data at higher saquinavir exposures (preliminary data from study up to 7200 mg per day) and the results of this study, no dose adjustments are necessary (discussed with medical officer).

Title: An open label study of the effect of rifampicin on the pharmacokinetics of Ro 31-8959 in healthy volunteers (Protocol WK 14436A)

Rifampin is commonly used to treat tuberculosis in AIDS patients, and is a known inducer of cytochrome p450 enzymes.

The pharmacokinetic interaction of saquinavir and rifampin at steady state was evaluated in 12 healthy males.

TREATMENT A: 600 mg saquinavir tid (8:00, 14:00 and 20:00 hours) for 7 days.
TREATMENT B: 600 mg saquinavir tid plus 600 mg rifampin QD for 7 days

All volunteers received treatment A in the first period and treatment B in the second period. Periods 1 and 2 were continued without any washout. Pharmacokinetics of saquinavir were determined on Day 7 (over 0-24 hours three oral doses were administered) of each treatment.

Results: Mean steady state plasma levels of saquinavir when administered with and without rifampin are provided in Figure 4. The following table summarizes the results:

Mean %CV (Range)	$Cmax_{0\text{-}24}$ ng/mL	Cmax Ratio	@$AUC_{0\text{-}24}$ ng.h/mL	@$AUC_{0\text{-}24}$ Ratio	Tmax (median)	#Cmin ng/mL
Saquinavir alone	144.5 41.0	....	1086 23.7		12	15.28 28.9
Saquinavir (with rifampin)	33.0 59.2	25% 56 (6%-46%)	187 43.1	17% 35 (7%-26%)	6	1.97 43.2

95% Confidence Intervals for AUC and Cmax *Treatment B versus Treatment A*				
	Mean Difference in Log values	*SE of Difference*	Mean*	95% C.I.**
AUC (0-24)	-0.79	0.052	16.1%	12.4 - 20.9
Cmax (0-24)	-0.67	0.085	21.0%	12.6 - 32.2

@ - Note AUC over 24 hour represents 3 doses. For each interval see attached table.
# - Cmin is defined as minimum concentration observed between 0 to 24 hours on Day 7 of each treatment.
* - Geometric mean calculated as exp (mean difference in log values).
** - Calculated as exp (mean diff in log ± (1.5% or 1.1) * SE of difference).

When comparing treatments A *and* B *over a 24 hour period,* it was found that AUC, Cmax and Cmin were significantly ($p<0.0001$) lower when saquinavir was co-administered with rifampin. Relative bioavailability (AUC) was about 16% and relative Cmax was about 21% when saquinavir and rifampin were concomitantly administered. Thus hepatic enzyme induction caused by rifampin markedly affected steady state pharmacokinetics of saquinavir.

This study was carried out in healthy volunteers. The effect of rifampin treatment on *enzyme induction and saquinavir pharmacokinetics* in patients is unknown. Saquinavir and rifampin (and other known or potential inducers of cytochrome p450) should not be concomitantly administered.

Interaction study between INVIRASE and rifabutin. (Preliminary report)

Thirteen HIV-infected males received saquinavir 600 mg tid alone or with rifabutin 300 mg qd in a sequential design as outlined below

Period I (Days 0-8): Saquinavir 600 mg tid for 7 days (morning dose only on Day 7)
Period II (Days 8-14): Saquinavir 600 mg tid + rifabutin 300 mg qd for 7 days (morning doses only on Day 14)
Period III (Days 15-21): Saquinavir 600 mg tid + rifabutin 300 mg qd for a further 7 days (morning doses only on day 21)

The following tables contain the mean (%cv) data from the study:

SAQUINAVIR	Period I	Period II	Period III
AUC_{0-24} (ng*h/mL)	1612 (57)	954 (47)	928 (63)
C_{max} (ng/mL)	377 (107)	200 (44)	238 (75)
$T_{1/2}$ (h)	9.7 (8.3)	10.1 (26.3)	11.1 (46)

RIFABUTIN	Period II	Period III
AUC_{0-24} (ng*h/mL)	3183 (45)	3547 (30)
C_{max} (ng/mL)	374 (50)	413 (31)
$T_{1/2}$ (h)	13.7 (34.6)	13.99 (55.6)

Ten of the 12 subjects had decreases in their 24 hour saquinavir AUC after coadministration with rifabutin (average 42% decrease; range 22-64%) and 2 subjects had higher AUC (8% and 18% increase). The data from this study demonstrate that rifabutin induces the metabolism of saquinavir resulting in lower plasma concentrations of saquinavir when the two drugs are given concomitantly.

f. GENDER

Study WK14340- A bioavailability study of a new suspension formulation of Ro 31-8959 as /043 (intended for paediatric use) relative to the standard capsule formulation /014 (Vol. 169)

Mean (%CV) data from 12 healthy volunteers (6 ♂, 6 ♀) who received 600 mg of *saquinavir* (3 x 200 mg) after a standardized breakfast.

Parameter	C_{max} (ng/mL)	T_{max} (h) median	AUC8 (µg*h/L)	AUCt* (µg*h/L)
All Subjects (n = 12)	82.4 (79.0)	4.5	156.7 (93.2)	169.6 (90.0)
Females (n = 6)	38.0 (63.3)	.....	.....	83.0 (45.0)
Males (n = 6) (n = 5)	126.8 (49.9) 105 (36.1)	.....	.....	256.1 (69.7) 186 (34.1)

*t = 12h or last measurable time

In this study, females had lower C_{max} and AUC values compared to males, however, the numbers of subjects were too low to perform a formal statistical analysis. There was one male who had much higher concentrations than the others in the group. When the values from this individual were left out, the mean AUC and C_{max} decreased.

Pooled gender analysis of data from either healthy subjects or patients did not reveal significant differences between males and females. The overall number of female study subjects was low.

3. IN VITRO STUDIES:

a. Protein binding

<u>Report W-141965, Vol 78, page 151</u>
The protein binding of 14-carbon labelled saquinavir was determined using equilibrium dialysis and ultrafiltration at 37°C. Plasma was harvested and pooled from the blood of healthy volunteers. Over a drug concentration range of 15-700 ng/mL, the protein binding averaged 98%.

b. Metabolism (hepatic microsomes)

<u>Report W-142069, Vol 88, page 212</u>
In order to identify the cytochrome P450 isozymes responsible for saquinavir metabolism, studies in pooled human liver microsomes were performed. Cytochrome P450 3A was identified as the primary isozyme responsible for saquinavir metabolism. This was demonstrated by inhibition of saquinavir metabolism in the presence of ketoconazole and midazolam (CYP 3A4 substrates). Incubations with compounds known to be substrates for other cytochrome P450 isozymes (furafylline, methoxycoumarin, coumarin, sulphaphenazole, tolbutamide, temazepam and p-nitrophenol) did not indicate that such isozymes were significantly involved in saquinavir metabolism.

Report W-142062, Vol 90, page 191

This study evaluated the ability of saquinavir to inhibit cytochrome P450 enzymes commonly involved in drug metabolism (2D6 and 2C9), but not involved in its own metabolism. The 2C9 hydroxylation of tolbutamide and the 2D6 mediated hydroxylation of bufuralol were not significantly inhibited by saquinavir, 3 μM.

c. Drug interactions (hepatic microsomes)

Report W-142117, Vol 90, page 174

The effect of saquinavir on the in vitro metabolism of selected compounds used in HIV-infected patients was studied in pooled liver microsomes from 5 male subjects (non HIV-infected). The compounds studied include: terfenadine, pyrazinamide, ergotamine, ketoconazole, nifedipine, and clindamycin. Saquinavir concentrations of 1, 3, and 10 μM were used in the microsomal incubations (done in triplicate), with 3 μM representing projected in vivo concentrations in the liver. There was no significant loss of dapsone or pyrazinamide from the microsomal incubations, therefore, the effects of saquinavir could not be determined. The following table summarizes the results for the other compounds studied.

% inhibition of compounds in the presence of saquinavir (mean of 3 incubations)					
Saquinavir Concentration (μM)	nifedipine	terfenadine	ergotamine	ketoconazole	clindamycin
1	24	0	31	17	29
3	62	0	29	20	68
10	83	14	46	53	84

A significant interaction was defined as greater than 50% inhibition. At the saquinavir concentration of 3 μM, a significant interaction was observed for nifedipine and clindamycin. It should be noted that in vitro studies serve as guides when deciding what in vivo drug interaction studies should be done. The presence or absence of an interaction in vitro does not guarantee that a similar result will be observed in vivo. Cytochrome P450 enzymes are located in the gastrointestinal tract as well as in the liver, however, the Applicant did not perform experiments using intestinal preparations.

B. PATIENTS (single/multiple dose, drug interactions):

STUDY- V13330- A randomized phase I-II double-blind study, to investigate the anti-viral activity, tolerability and pharmacokinetics of varying doses of oral Ro 31-8959 (HIV proteinase inhibitor) in combination with a fixed dose of zidovudine (ZDV) administered to previously untreated symptomatic HIV-infected individuals. (Vols. 240-248, dates 2/92-2/93)

This 16 week study examined 1) dose response relationships, 2) combination versus monotherapy, 3) tolerability of chronic orally administered Ro 31-8959 (Saquinavir), 4) pharmacokinetics, 5) pharmacokinetic interaction between saquinavir and ZDV, 6) correlation of pharmacokinetic parameters and anti-viral activity and 7) the potential reduction in HIV infectivity of saquinavir therapy, using quantitative virus titration and PCR technology. Ninety-two HIV positive patients (♂♀) with CD4 cell counts ≤ 300, age 18-65, received treatment and 80 completed the study. The reasons for the dropouts will not be detailed but were generally a result of noncompliance or adverse events. Patients were randomly assigned to receive the following treatments:

	Saquinavir	ZDV	n
1.	Placebo tid	200 mg tid	17
2.	75 mg tid	200 mg tid	18
3.	200 mg tid	200 mg tid	18
4.	600 mg tid	200 mg tid	20
5.	600 mg tid	Placebo tid	19

Medications were taken within 30 minutes after eating a meal. Pharmacokinetic assessments were made on Day 7 and on Day 42 ± 4, with samples being collected prior to dosing and at 1, 2, 3, 4, 6 and 8 hours after dosing. Single blood samples were collected during weeks 8 and 12 (3 hours after dosing) and during week 16, prior to dosing and at 1, 3 and 6 hours after dosing. Saquinavir samples were analyzed by Huntingdon Research Centre and ZDV,ZDVG samples were analyzed by the Wellcome Foundation. The following table contains the pharmacokinetic parameter estimates for saquinavir and a figure of the mean concentration versus time profile follows. The profiles were obtained either in week 1 or 6 or during the extension phase of the study.

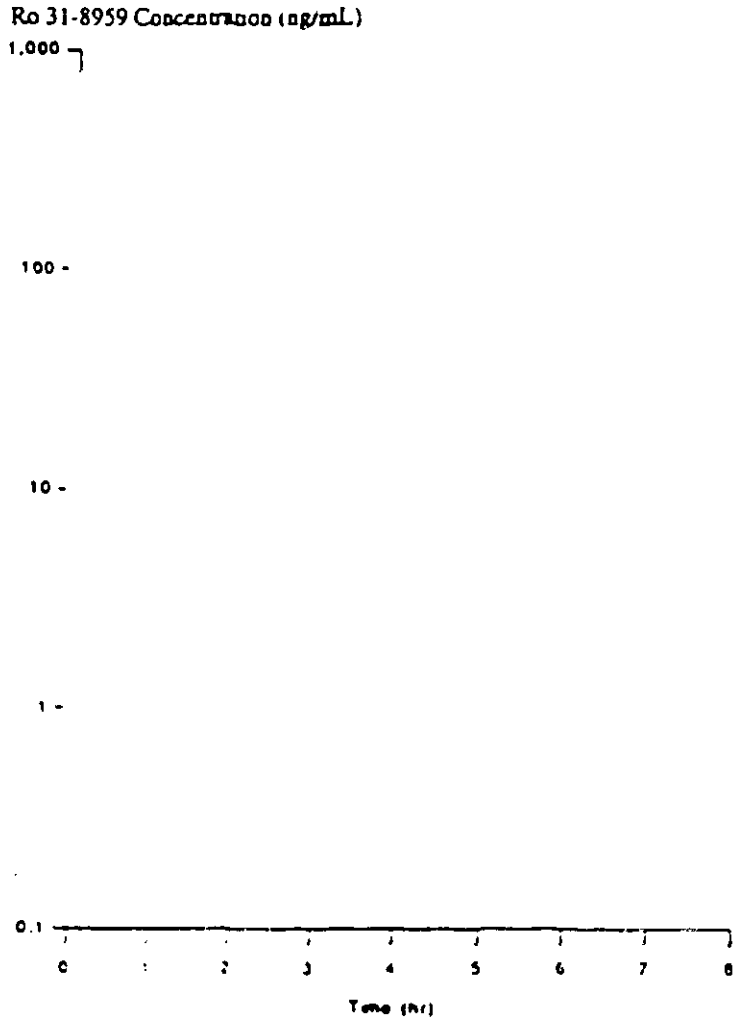
Mean (%CV) Saquinavir Pharmacokinetic Parameters After Multiple Dosing				
Parameter	75 mg + ZDV	200 mg + ZDV	600 mg + ZDV	600 mg alone
C_{max} (ng mL)	10.3 (78.4)	56.7 (110.1	199.9 (92.3)	270.0 (108.8)
n	17	18	20	19
T_{max} (hr)*	3.0	3.0	3.0	3.0
n	16	18	20	19
T_{lag} (hr)*	0.5	0.0	0.0	0.0
n	16	18	19	18
AUC$_{8}$ (ng*hr/mL)	21.5 (62.9)	18.2 (54.6	114.7 (79.0)	73.9.4 (89.8)
n	16	15	20	18

*Median

The Applicant performed linear regression analyses between AUC_8 and levels of SGOT, SGPT, alkaline phosphatase, bilirubin, albumin and creatinine, as well as age, bodyweight and gender. No apparent trends were found. AUC_8 and Cmax increased more than proportionately with dose. There was a large degree of intersubject variability. Concentrations of saquinavir in pre-dose and 8 hour samples were not significantly different for any of the treatments, indicating the attainment of steady state. The log transformed parameter estimates after the 600 mg saquinavir doses in the presence and absence of ZDV were compared by an one-way analysis of variance and no statistically significant differences were found for C_{max} or AUC_8.

The Applicant states that it was not possible to extract data on variation of pharmacokinetic parameters within patients because in most patients, at least one of the profiles at weeks 1 and 6 were unusable as a second dose of medication had been taken within the dose interval.

Figure 9. Mean Plasma Concentration of Ro 31-8959 in Patients after Multiple Oral Dosing of Ro 31-8959 Alone or in Combination with ZDV



Profiles on the median data (not shown) are qualitatively similar to those of the mean data shown above

The following tables and figure contain mean pharmacokinetic parameter estimates and concentration vs time profiles for ZDV and ZDVG.

Mean (%CV) ZDV Pharmacokinetic Parameters After Multiple Dosing				
Parameter	ZDV + 75 mg Saquinavir	ZDV + 200 mg Saquinavir	ZDV + 600 mg Saquinavir	ZDV alone
C_{max} (nmol/mL) n	2.9 (38.7) 17	3.0 (78.0) 17	2.6 (33.6) 18	2.7 (43.0) 16
T_{max} (h)* n	1.0 17	1.0 17	1.0 18	1.0 16
AUC8 (nmol*h/mL) n	6.7 (36.8) 17	6.8 (47.1) 17	6.3 (34.0) 18	6.8 (48.1) 16

*Median

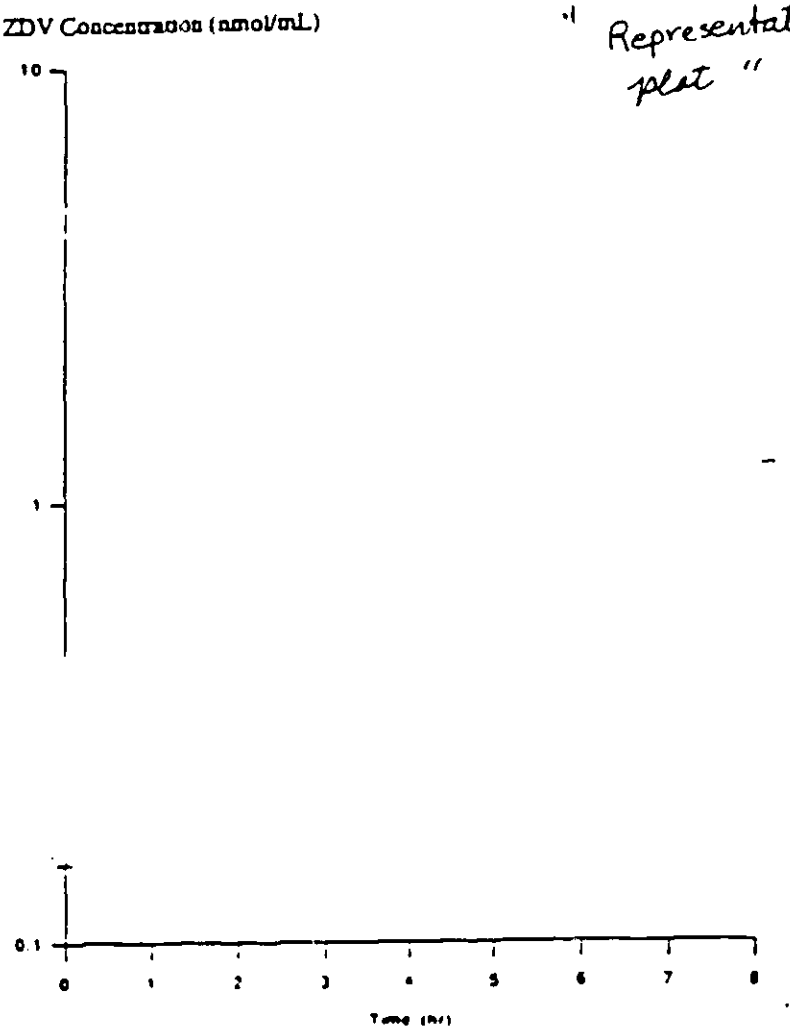
Mean (%CV) ZDVG Pharmacokinetic Parameters After Multiple Dosing				
Parameter	ZDV + 75 mg Saquinavir	ZDV + 200 mg Saquinavir	ZDV + 600 mg Saquinavir	ZDV alone
C_{max} (nmol/mL) n	8.7 (30.7) 17	9.1 (33.1) 17	9.8 (33.7) 18	8.2 (34.3) 16
T_{max} (h)* n	2.0 17	2.0 17	1.0 18	2.0 16
AUC8 (nmol*h/mL) n	20.8 (27.0) 17	21.9 (30.9) 17	21.9 (27.0) 18	20.4 (36.6) 16

*Median

Pharmacokinetic parameters for ZDV and ZDVG were not affected by coadministration with saquinavir as evidenced by similar parameters over all treatment groups.

Figure 10. Mean Plasma Concentration of ZDV in Patients after Multiple Oral Dosing of 200 mg ZDV Alone or in Combination with Increasing Doses of Ro 31-8959

"Representative of ZDVG plot"



Profiles of the median data (not shown) are qualitatively similar to those of the mean data shown above

The pharmacokinetic-response relationship analysis examined β2 microglobulin, neopterin, CD4, p24 and antigen in relation to AUC8 and log AUC8 (base10 log). A significant relationship was only established for changes in neopterin and log AUC8.

This study demonstrated that steady state is achieved by 1 week of dosing with saquinavir. It demonstrated greater than proportionate increases in AUC and C_{max} with dose. This study demonstrated that a significant pharmacokinetic interaction does not occur for either drug when saquinavir is coadministered with ZDV. This study further demonstrated that the pharmacokinetics of saquinavir is subject to a large degree of intersubject variability. The Applicant did not correlate adverse events to drug exposure, however adverse events were not generally serious.

STUDY V-13329- A randomized phase I-II double-blind study to investigate the anti-viral activity, tolerability and pharmacokinetics of oral Ro 31-8959 (HIV proteinase inhibitor) in previously ZDV treated HIV-infected individuals, treated at doses of 75, 200 and 600 mg thrice daily. (Vols. 233-239, dates 12/91-9/92)

Sixty-one HIV infected patients (♂ ♀), age 19 to 64 years, were enrolled in this double-blind parallel group study which lasted 16 weeks, and 58 completed the study. Patients were randomly assigned to receive the following treatments:

	Saquinavir	ZDV	n	♂/♀
1.	75 mg tid	200 mg tid	20	15/5
2.	200 mg tid	200 mg tid	21	14/7
3.	600 mg tid	200 mg tid	20	16/4

Medications were taken within 30 minutes after eating a meal (meals were not standardized). Pharmacokinetic assessments were performed on Day 1 and on Day 28, with samples being collected prior to dosing and at 0.5, 1, 1.5, 2, 3, 4, 6, 7 and 8 hours after the first dose. Single blood samples were collected during weeks 8 and 12. During week 16, samples were collected prior to dosing and 1, 3 and 6 hours after dosing. Saquinavir samples were analyzed by . The following table contains the pharmacokinetic parameter estimates for saquinavir and a figure of the mean concentration versus time profiles follows as well.

Mean (%CV) Saquinavir Pharmacokinetic Parameters Day 1			
Parameter	75 mg tid	200 mg tid	600 mg tid
Cmax (ng/mL)	9.8 (111.9)	40.7 (85.6)	161.3 (72.9)
n	16	20	20
Tmax (h)*	1.5	3.0	3.0
n	16	20	20
T½ (h)*	0.5	0.5	2.4
n	16	20	20
AUC8 (µg*h/L)	16.3 (133.7)	75.4 (85.9)	386.2 (89.1)
n	16	19	20

Mean (%CV) Saquinavir Pharmacokinetic Parameters Day 28			
Parameter	75 mg tid	200 mg tid	600 mg tid
Cmax (ng/mL)	20.9 (96.3)	85.0 (67.1)	242.3 (75.9)
n	17	19	19
Tmax (h)*	3.0	2.0	2.0
n	17	19	19
T½ (h)*	0.5	0.5	0.5
n	17	18	19
AUC8 (µg*h/L)	47.95 (95.8)	207.3 (58.1)	667.2 (77.3)
n	17	19	19

Mean (%CV) Saquinavir Pharmacokinetic Parameters Week 16 Based on samples collected 0, 1, 3 and 6 hours after dosing			
Parameter	75 mg tid	200 mg tid	600 mg tid
Cmax (ng/mL)	9.3 (136.0)	48.3 (80.4)	142.7 (83.8)
n	17	19	20
Tmax (h)*	3.0	3.0	3.0
n	17	19	20
AUC8 (µg*h/L)	27.5 (72.3)	151.1 (65.4)	490.2 (82.0)
n	13	19	20

*Median

Greater than proportional increases in mean AUC and Cmax were observed when the 75 mg dose was compared to the others. However, when the 200 mg dose was compared to the 600 mg dose, directly proportional increases in mean AUC and Cmax were observed. This may be indicative of differences in the 75 mg capsule preparation relative to the 200 mg capsule preparation which was used for the two higher doses. Significant differences were observed between Day 1 and Day 28 AUC8 values. The percentage accumulation was 250% (90% CI, 117-465%), 201% (129-294%) and 102% (30.1-213%) for the 75 mg, 200 mg and 600 mg tid doses respectively. The AUC8 values at steady state from this study are comparable to those in study V13330 for the 200 and 600 mg doses.

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The intra-subject variability in steady state exposure (intra-subject s.d./overall mean x 100) was estimated by comparing the AUC of the simplified profile of week 16 with the AUC from Day 28 calculated from the same set of sampling times. Comparison of the truncated profiles showed no significant differences. The intra-subject variability in steady state exposure was 90%, 51% and 62%, respectively, for the 75 mg, 200 mg and 600 mg dose groups. Inter-subject variability was high among all groups as well, as indicated by the high %CV. No significant relationships were found between AUC at Day 28 (ss) and gender, body weight or biochemical indices of liver function for all dosages.

A significant linear relationship was observed between the change in CD4 counts from baseline and AUC for all weeks except week 16. When logAUC was used, the linear regressions were better and were significant even at week 16. The following equation was used as the basis for the pharmacodynamic model: $\Delta CD4 = Int + Grad * logAUC$. The intercept (Int) and gradient (Grad) were different at different visits during the study. According to the Applicant, the changing value of the intercept represents the change in CD4 counts ($\Delta CD4$) which occurs in the absence of treatment, whilst the changing value of the gradient represents the changing influence of exposure to saquinavir over the course of the study. The Int and Grad were plotted against time to try and develop a model to describe how they change over time. An exponential model best described the behavior of the Int over time and a cumulative exponential was used to describe the behavior of the Grad. The final model used all of the data simultaneously using NONMEM, with an additive intra-individual error structure, and is described below.

$$\Delta CD4 = Int + Grad * logAUC$$

where $Int = A(\exp(-B*t)-1)$

and $Grad = C(1-\exp(-k*t))$

The model yielded the following results
A = 84.3, B = 0.264, C = 38.6, K = 0.372, error = 40.

The model predicts a maximal increase in CD4 of 32 cells/μL for an AUC of 700 μg*h/L, which occurs on day 7. According to the Applicant, this is in agreement with the reported median change seen at 1 week in the 600 mg dose group, which had a mean AUC of 719 μg*h/L. Figures demonstrating the relationship between Int/Grad and time, as well as predicted versus modelled changes in CD4 as a function of logAUC and time follow.

A linear relationship was found for Δlog(p24 antigen) and logAUC so this relationship was used as the basis for model development. In developing the model no apparent dependency of the intercepts and gradients for the individual weeks was established. The relationship between Δlog(p24 antigen) was examined individually for the effects of time and logAUC. The linear regressions were statistically significant, however, less than 10% of the variation in p24 antigen values was explained. This analysis showed that there was little difference in effect between doses and that time had little, if any, effect on Δlog(p24 antigen). This result is based on a limited amount of data and does not necessarily reflect what the outcome would be under a different set of circumstances.

There was no apparent relationship between dose and adverse events which were felt to be related to treatment.

Study NV14255E/ACTG 229 - Phase II, double-blind, randomized study of Ro 31-8959-Zidovudine versus Zidovudine-Zalcitabine versus Ro 31-8959-Zidovudine-Zalcitabine. (Vols.181- 194, dates 3/93-12/93)

Three hundred two previously ZDV exposed patients (CD4 between 50 and 300), age 19-75 years, were enrolled in this study, and were similarly randomized to one of the three treatment groups. All patients did not complete the study. The reasons for withdrawals will not be detailed but have been reviewed and are acceptable. The objectives of this study were to evaluate immunologic activity, reduction in HIV plasma viremia , safety and tolerability and pharmacokinetics of combination therapy. Patients received the following treatments for 24 weeks with opportunity for an extension:

	ZDV	Saquinavir	ddC	n	♂/♀
1.	200 mg tid	placebo	0.75 mg tid	100	94/6
2.	200 mg tid	600 mg tid	placebo	99	87/12
3.	200 mg tid	600 mg tid	0.75 mg tid	98	98/9

Drugs were taken simultaneously within 30 minutes after food intake. The doses for the ZDV/ddC combination were chosen in accordance with the ddC package insert. Intensive pharmacokinetic sampling was performed at 3 of the study centers at weeks 1 and 12. For these profiles, samples were collected prior to dosing and 1, 2, 3, 4, 6 and 8 hours after dosing, from a maximum of 35 patients from each center. Urine was also collected over the 8 hour dosing interval. All patients at all centers had single blood samples collected during weeks 1, 12 and 24 for population pharmacokinetic evaluations. Blood samples were to be collected at the time of any serious adverse event which caused treatment to be interrupted and also in patients receiving rifabutin, however, there was no occasion to collect such samples. Patients were evaluable for the pharmacokinetic assessments if they had at least one baseline and one post treatment sample obtained. If they had 4 or more samples obtained during the 8 hour dose interval, they were included in the intensive pharmacokinetic analyses. Patients were evaluable for the population pharmacokinetic analysis if they had at least one sample collected during the 24 week study period.

Log_{10} transformed plasma concentration data from pre-dose and 8 hour samples were compared by a paired t-test. Concentrations were not significantly different in the samples collected at the two times, suggesting that 1) steady state was achieved by 1 week and 2) the extended interval between the evening and morning doses does not significantly impact on measured C_{min} concentrations. The following table contains mean (%CV) pharmacokinetic parameter estimates for saquinavir. Statistically significant differences between treatments and weeks were not observed for saquinavir pharmacokinetic parameters.

SAQUINAVIR	Saquinavir + ZDV				Saquinavir + ZDV + ddC			
Week	Cmax (ng/mL)	Tmax (h)	Tlag (h)	AUC8 (µg*h/L)	Cmax (ng/mL)	Tmax (h)	Tlag (h)	AUC8 (µg*h/L)
1	177 (70) n = 18	5 n = 18	1 n = 17	633 (60) n = 18	213 (105) n = 26	3 n = 26	0 n = 26	686 (89) n = 27
12	135 (61) n = 16	3 n = 16	1 n = 13	531 (54) n = 19	201 (83) n = 24	4 n = 24	1 n = 23	765 (82) n = 24

The data from week 1 were used to determine if there was a linear correlation between Cmax and AUC8. Significant correlations were seen in both treatment groups, having r^2 values of 0.94 and 0.90 ($p < 0.0001$). Because of the observed linear relationship between these 2 parameters, the Applicant only used AUC8 in their subsequent detailed analyses. The effect of age, weight and CD4 count at screening was regressed against AUC8 at week 1 and only age showed a statistically significant predictive trend ($p = 0.031$) of increasing AUC8 with age (see following figure). There were not sufficient numbers of females and non-caucasians to investigate the influence of gender or race.

Three patients who underwent intensive pharmacokinetic sampling were taking concomitant medications known to induce or inhibit CYP3A4 activity. One received ketoconazole and rifampin during week 1 but only ketoconazole during week 12, while two other patients received ketoconazole during weeks 1 and 12. When the AUC8 and Cmax values from these patients were compared to the group means, they were within the ranges observed for the other study patients

Note- Saquinavir concentrations were measured in some samples from patients who were assigned to the ZDV-ddC treatment group (15 samples from 11 patients). It was determined that one patient was administered saquinavir instead of placebo and another was given the triple combination instead of the double combination during week one, but was later given the correct treatment. Four patients had very low levels of saquinavir, which could have been attributed to sample contamination, however, samples from the remaining five patients had concentrations which were too high to be attributed to contamination (~ 50-450 ng/mL). It is possible that there was some exchanging of capsules amongst patients.

PK/PD Analysis - A linear regression model was used to determine the relationship between NAUC of the normalized CD4 count (value greater than 1 indicates a net increase in CD4 count over the 24 week period) over the 24 weeks of the study and AUC8 from week 1. For the saquinavir-ZDV group there was no significant correlation (slope = -0.0001, $r^2 = 0.0440$, $p = 0.40$). For the saquinavir ZDV-ddC group there was a significant correlation (slope = -0.0004, $r^2 = 0.2364$, $p = 0.01$). The significance of this relationship is not clear since the pharmacokinetics of saquinavir was not different between study groups and the response was. It may not be appropriate to compare only saquinavir in the model since the other drugs are also assumed to have an effect. It is not clear how the effect of the other drugs can be separated out from the effect of saquinavir (intercepts?). This comparison differs from that reported earlier for another study which compared logAUC.

The population modeling will be submitted in a subsequent report.

3.3.3.8. *Effect of Covariates Age, Weight and CD_4 on AUC_8 and C_{max} for Ro 31-8959*

The effect of age, weight and CD_4 at screening were investigated by regression of these covariates against AUC_8 at week 1 (Appendix XXXVIII). Only age (Ro 31-8959-ZDV group) was found to show any statistically significant ($p=0.031$) predictive trend for observed AUC_8 (Figure 8 and Figure 9). There were insufficient numbers of non-caucasians and females in the pharmacokinetic population to investigate the influence of race or sex on the AUC_8.

Figure 8. Linear Regression of Patient's Age at Baseline Against Observed AUC_8 for Ro 31-8959 at Week 1 for Patients Allocated to Ro 31-8959-ZDV

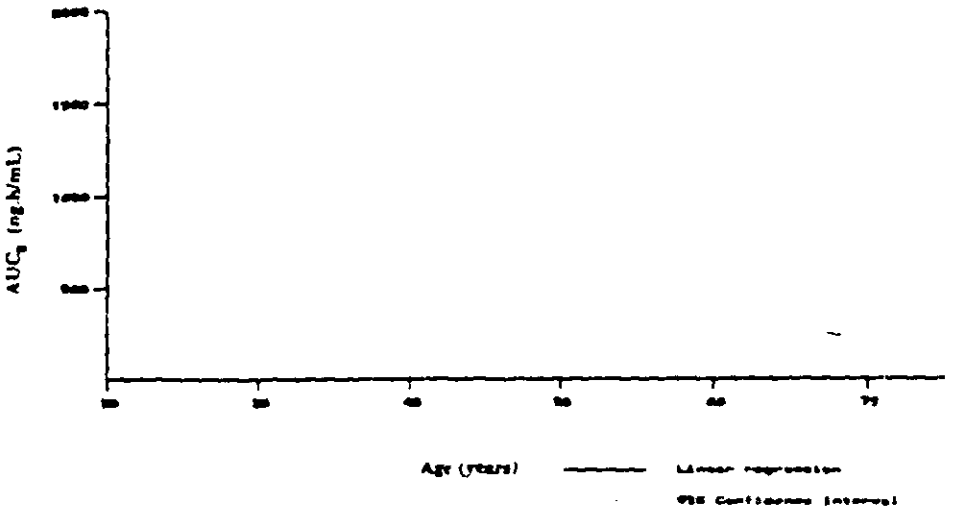
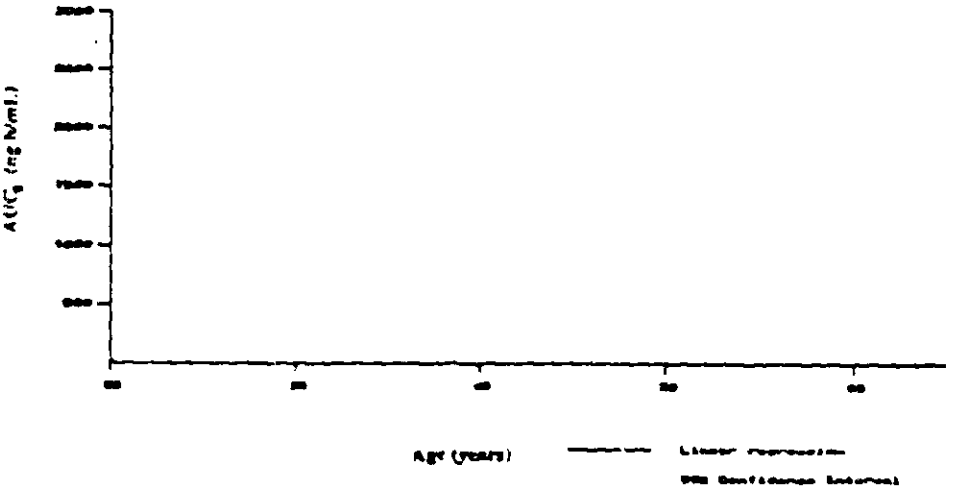


Figure 9. Linear Regression of Patient's Age at Baseline Against Observed AUC_8 for Ro 31-8959 at Week 1 for Patients Allocated to Ro 31-8959-ZDV-ddC



ZDV - The following table contains mean (%CV) pharmacokinetic parameters for ZDV. Statistical analysis of the $\log_{10}$ transformed data revealed that ZDV pharmacokinetics was not different among all treatments, and for patients having measurements at both week 1 and 12, there was no difference between the two days.

ZDV	ZDV + ddC			Saquinavir + ZDV			Saquinavir + ZDV + ddC		
Week	Cmax (ng/mL)	Tmax[a] (h)	AUC8 (µg*h/L)	Cmax (ng/mL)	Tmax (h)	AUC8 (µg*h/L)	Cmax (ng/mL)	Tmax (h)	AUC8 (µg*h/L)
1	434 (43) n=25	2 n=25	914 (35) n=25	421 (48) n=18	1.5 n=18	1010 (49) n=18	499 (56) n=27	1.3 n=27	1010 (43) n=27
12	370 (41) n=22	2 n=22	1030 (44) n=22	368 (48) n=19	2 n=19	924 (48) n=19	433 (60) n=24	2 n=24	1020 (45) n=24

a Median

The following table contains mean (%CV) renal clearance (mL/min) values for ZDV during the various treatments, using all available data. The Applicant notes that the high %CV for the ZDV + ddC group resulted from one subject who had extremely high renal clearance (3950 mL/min, which is greater than renal blood flow). No explanation could be found for the high value so it was not excluded.

Renal Clearance mL/min			
Week	ZDV + ddC	Saquinavir + ZDV	Saquinavir + ZDV + ddC
1	388 (194) n=25	310 (77) n=18	314 (99) n=27
12	212 (74) n=19	230 (50) n=18	232 (47) n=24

The following table contains a comparison of mean renal clearance of ZDV between weeks 1 and 12, using data from patients who had measurements on both occasions.

Renal Clearance mL/min			
Week	ZDV + ddC	Saquinavir + ZDV	Saquinavir + ZDV + ddC
1	452	324	313
12	212	236	232

ZDV renal clearance appeared to decrease from week 1 to week 12, however this decrease was not statistically significant

ddC - The following table contains mean (%CV) parameter estimates for ddC. AUC was

not significantly different among the treatment groups. The mean C_{max} at week 12 was slightly, but statistically significantly, lower in the 2 treatment group versus the 3 treatment group. For patients having data for weeks 1 and 12, there was no significant difference in the pharmacokinetic parameters between the two weeks.

ddC	ZDV + ddC			Saquinavir + ZDV + ddC		
Week	C_{max} (ng/mL)	T_{max} (h)	AUC8 (µg*h/mL)	C_{max} (ng/mL)	T_{max} (h)	AUC8 (µg*h/mL)
1	6.69 (31) n = 25	2 n = 25	28.3 (29) n = 25	7.16 (29.8) n = 27	2 n = 27	28.8 (28) n = 27
12	6.18 (35) n = 19	2 n = 19	26.5 (31) n = 19	7.58 (27) n = 24	2 n = 24	30.7 (27) n = 24

The following table contains the mean (%CV) renal clearance (mL/min) values for ddC. Statistically significant differences were not found between the weeks or treatment groups.

ddC Renal Clearance mL/min		
Week	ZDV + ddC	Saquinavir + ZDV + ddC
1	241 (33) n = 25	208 (42) n = 27
12	192 (45) n = 16	201 (29) n = 24

The pharmacokinetic parameters determined for ddC in the present study are comparable to historical values.

This study demonstrated that concomitant administration of saquinavir, ZDV and ddC does not result in significant pharmacokinetic interactions.

Study O-13328: A randomized phase I-II double-blind study to assess the anti-viral activity, tolerability and pharmacokinetics of oral Ro 31-8959 (HIV proteinase inhibitor) in previously untreated HIV-infected individuals, either minimally symptomatic or with asymptomatic disease and at risk of disease progression, treated at doses of 25, 75, 200 and 600 mg thrice daily. (Vol. 232; Dates 8/91-11/92, UK)

Forty-nine males, age 21-55 years, participated in this 16 week study (plus 1 month extension) and received saquinavir doses of 25, 75, 200 and 600 mg tid. Doses were taken within 30 minutes of food ingestion. Dose response relationships and pharmacokinetics were determined. Pharmacokinetic profiles were obtained on Days 1 & 28 and additional samples were also collected at weeks 8, 12 and 16. Samples for the pharmacokinetic profiles were collected prior to dosing and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7 and 8 hours after dosing.

Patients who received the 25 mg regimen generally had saquinavir concentrations below

the 0.5 ng/mL limit of quantitation, therefore, pharmacokinetic parameters could not be determined. Mean (%CV) parameter estimates for the other doses are in the following tables. There were greater than proportional increases in C_{max} and AUC as dose increased. The % accumulation from Day 1 to 28 was 108% (90%CI = 32-230%) and 252% (90% CI = 93-543%) for the 200 and 600 mg doses, respectively.

Mean (%CV) Saquinavir Pharmacokinetic Parameters Day 1			
Parameter	75 mg tid	200 mg tid	600 mg tid
C_{max} (ng/mL) n	7.17 (111) 11	17.99 (73.9) 12	83.8 (95.4) 12
T_{max} (h)* n	1.5 11	3.5 12	4 12
T_{lag} (h)* n	1 11	0.5 12	0.5 12
AUC8 (ug*h/L) n	not calculable	38.0 (48.2) 12	228.9 (115.5) 12

Mean (%CV) Saquinavir Pharmacokinetic Parameters Day 28			
Parameter	75 mg tid	200 mg tid	600 mg tid
C_{max} (ng/mL) n	8.39 (55.2) 11	33.7 (74.1) 13	196.4 (51.3) 11
T_{max} (h)* n	1.5 11	3.0 13	2 11
T_{lag} (h)* n	not calculable	0.5 11	0.5 11
AUC8 (ug*h/L) n	17.0 (72.3) 11	88.1 (69.6 13	611.9 (67.4) 11

Study EV14757 - An exploratory phase I/II dose escalating study of Ro 31-8959 (HIV proteinase inhibitor) in patients with HIV disease. (Vol. 176)

Note: An interim study report was submitted. The data have not been audited.

This study was intended to determine if increasing the dose of saquinavir, and presumably systemic exposure, would further decrease plasma viremia over that seen in other trials. If a benefit of higher drug exposure was established, the Applicant would be encouraged to develop a formulation with increased bioavailability such that smaller numbers of capsules would be required per dose. This was an open label, non-randomized, single center, 2 dose parallel, ascending study in patients with intermediate HIV disease (CD4 200-500) who were naive to proteinase and could have had up to 12 weeks of ZDV treatment. Patients were dosed with saquinavir 600 or 1200 mg q4h six times per day (n = 20 per group), after food. Activity was assessed by measuring change in CD4 count from baseline, reduction in serum p24 antigen levels, development of resistance as implied by mutations at HIV

proteinase codons 48 and 90, pharmacokinetics and reduction in viral burden by using plasma quantitative RNA polymerase chain reaction and quantitative viral culture. RNA PCR (virologic) and CD4 lymphocyte count (immunologic) were used as the primary parameters for efficacy. The report submitted to the NDA summarizes data from 20 patients (19 ♂, 1 ♀) who received the lower dose (3600 mg/day). The analysis covers the primary treatment period of 24 weeks and includes data from the primary efficacy parameters.

Pharmacokinetic assessments were to be made in the first 8 patients of each treatment. Blood samples (5 mL) were collected on Days 1 & 112 (week 16) prior to the first morning dose and 1, 2, 4, 5, 6, 8, 9, 10 and 12 hours after the first morning dose. On Day 28, samples were collected prior to the morning dose and 1, 2, 4, 5, 6, 8, 9, 10, 12, 13, 14, 16, 17, 18, 20, 21, 22 and 24 hours after the first morning dose. Patients were given food prior to the first morning dose of saquinavir. An RIA assay was used to analyze the samples. A quality assurance check had not been performed at the time of submission. Noncompartmental pharmacokinetic analysis was performed. The relationship between drug exposure and efficacy parameters of CD4 and RNA was examined by linear regression with the 24 hour AUC on Day 28.

SAQUINAVIR 600 MG Q4H n = 8, mean (%cv)			
Parameter	Day 1	Day 28	Day 112
AUC_4 (µg*h/L)	110.4 (88)	.....	
AUC_{12} (µg*h/L)	714.2 (60)	.....	1133 (43)
AUC_{24} (µg*h/L)	.....	2233 (35	.....
C_{ave} (ng/mL)	.....	93.1 (35)	94.4 (43)
C_{max} (ng/mL)	162.6 (52)	304.9 (39	183.1 (37)
T_{max} (h)	9	21	9
C_{min} (ng/mL)	.....	20.4 (49	39.1 (46)
T_{min} (h)	.....	1	5

During the review process, data from patients who received the higher dose (7200 mg/day) were submitted, and are summarized below

SAQUINAVIR 1200 MG Q4H n = 7, mean (%cv)			
Parameter	Day 1	Day 28	Day 112
AUC_4 (µg*h/L)	722.0 (88)	.....	
AUC_{12} (µg*h/L)	4092 (74)	.....	5983 (61)
AUC_{24} (µg*h/L)	.....	9742 (48)	.....
C_{ave} (ng/mL)	.....	405.9 (48)	[illegible]
C_{max} (ng/mL)	965.0 (73)	1204 (78)	1369 (97)
T_{max} (h)	1.1	1.7	1.7
C_{min} (ng/mL)	.....	123.5 (67)	151.9 (62)
T_{min} (h)	.....	1.0	3

A significant correlation was observed between saquinavir AUC and decreases in RNA. Although the data from this study are limited, they do support the idea that clinical benefit may be increased if higher concentrations of saquinavir can be achieved in patients.

VI. DISSOLUTION:

The Applicant evaluated the dissolution of saquinavir in the following five media: water, water/ethanol (80:20), citrate buffer pH 3, citrate buffer pH 5 and acetate buffer pH 4.4. These media were chosen based on the solubility of saquinavir described in the table below. In most cases, tests were performed on capsules from the batch used in the pivotal bioequivalence study (Batch PT 9238 B52). Tests used

Solvent	Solubility at 25°C (g/100mL)	Solubility at 37°C (g/100mL)
Water	0.222	0.270
Water/Ethanol (80:20)	0.398	0.610
0.1M HCl	0.0075	nd
0.1M NaOH	0.0017	nd
Citrate Buffer pH 3	0.222	0.307
Acetate Buffer pH 4	0.308	0.616
Citrate Buffer pH 5	0.169	nd
Artificial Intestinal Fluid (pH 7.5)	0.0027	nd

* Not determined

Citrate buffer pH 5, acetate buffer pH 4 and water were not deemed acceptable because complete dissolution by either method, with a highest mean ≥ Citrate buffer pH 3 was chosen over water/ethanol as it was felt to be more representative of in vivo conditions. Dissolution profiles were practically superimposable for the two methods (basket, paddle). The following table contains dissolution data generated from the batches used in the pivotal bioequivalence trial. For the reference, the values in brackets[] represent initial release results from 6 capsules, while the values above were generated after ROC210 was selected as the biobatch. The dissolution profiles are plotted in the following figure.

Method =

Mean ± SD % Dissolved (Range)	/014 Reference ROC210 (n = 12)	:622 Market PT 9238 852 (n = 6 - 6)	014 Welwyn PT 9238 855 n = 6 - 6)	Deuterated WEL019501 (n = 6)
10 minutes				
20 minutes				
30 minutes				
40 minutes				
45 minutes				
60 minutes				

The Applicant is proposing a dissolution method using
ssolved at 45 minutes The data in the above table indicate that the dissolution profile for the marketed formulation is lower than that for the reference, however, these formulations were demonstrated to be bioequivalent. It should also be noted that the release dissolution for the reference was lower than that determined at a later time.

The dissolution method proposed by the Applicant is acceptable, however, the data support a higher Q value than proposed by the Applicant It is concluded that the data support a specification of Q in 45 minutes.

2 pages
purged

Table

&

Figures

Table 3. Mean Pharmacokinetic Parameters for Each Dosing Interval of Ro31-8959 After Multiple Oral Dosing in the Presence and Absence of Rifampicin

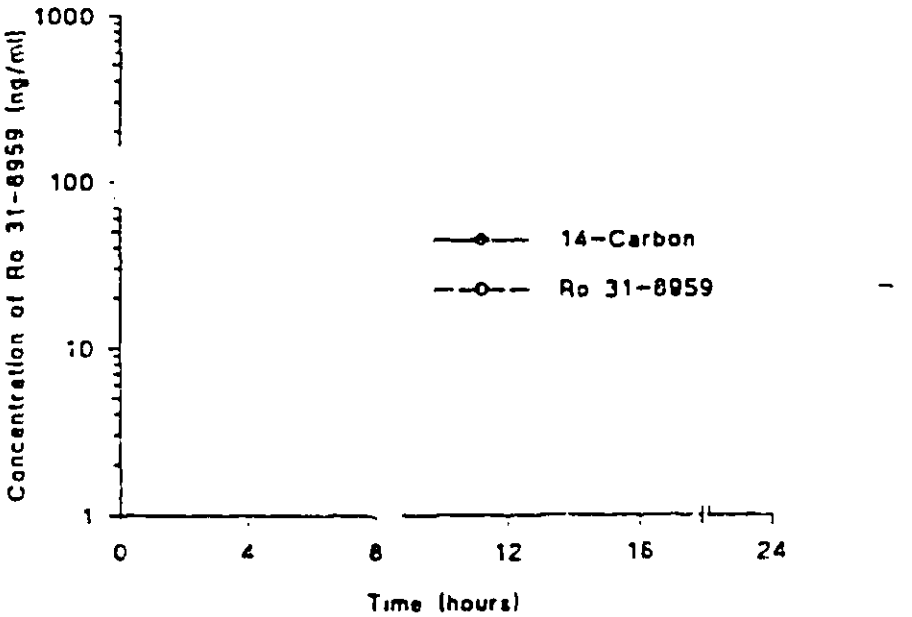
Treatment A: 600 mg Ro 31-8959 [illegible] d. for 7 days
Treatment B: 600 mg Ro 31-8959 [illegible] d. plus 600 rifampicin q.d. for 7 days.

	Treatment A		Treatment B	
	Mean	% CV	Mean	% CV
Cmax (ng/mL)				
0 - 6 h	20.15	39.1	3.41	61.7
6 - 12 h	114.39	35.2	15.35	60.4
12 - 24 h	89.05	59.9	8.59	56.2
tmax* (h)				
0 - 6 h	6		6	
6 - 12 h	12		12	
12 - 24 h	13		13	
Cmin (ng/mL)				
0 - 6 h	16.73	32.2	3.23	47.4
6 - 12 h	25.83	37.1	4.23	41.0
12 - 24 h	17.32	23.6	1.97	43.3
tmin* (h)				
0 - 6 h	1		1	
6 - 12 h	10		9	
12 - 24 h	20		21	
AUC (ng h/mL)				
0 - 6 h	266.2	35.9	21.3	51.6
6 - 12 h	342.8	46.2	37.6	49.9
12 - 24 h	477.1	21.1	37.3	46.2
Peak trough ratio Cmax/Cmin				
0 - 6 h	5.4		6.2	
6 - 12 h	5.6		4.3	
12 - 24 h	5.0		4.5	

*tmax and tmin values are presented as medians

Figure 1

Figure 1 Mean plasma levels of radioactivity and Ro 31-8959 after a 60 minute intravenous infusion of 29 µCi (10.5 mg) of the 14-carbon labelled drug



After oral administration the difference between radioactivity and Ro 31-8959 was much greater: average plasma concentrations at 1 and 10 hours *post* dose were 45 and 68 ng equivalents/mL for radioactivity and only 5.5 and 3.3 ng/mL for Ro 31-8959 (Tables A.III.3 and A.III.4).

Figure 2 Mean plasma levels of radioactivity and Ro 31-8959 after oral administration of 26 µCi (600 mg) of a suspension of the 14-carbon labelled drug

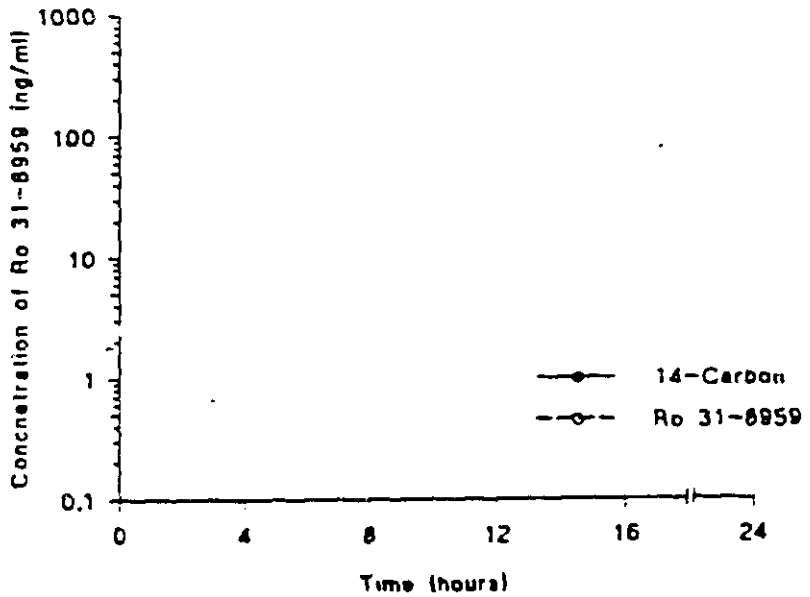


Figure 1: Mean Plasma Concentration-Time Profiles of Ro 31-8959

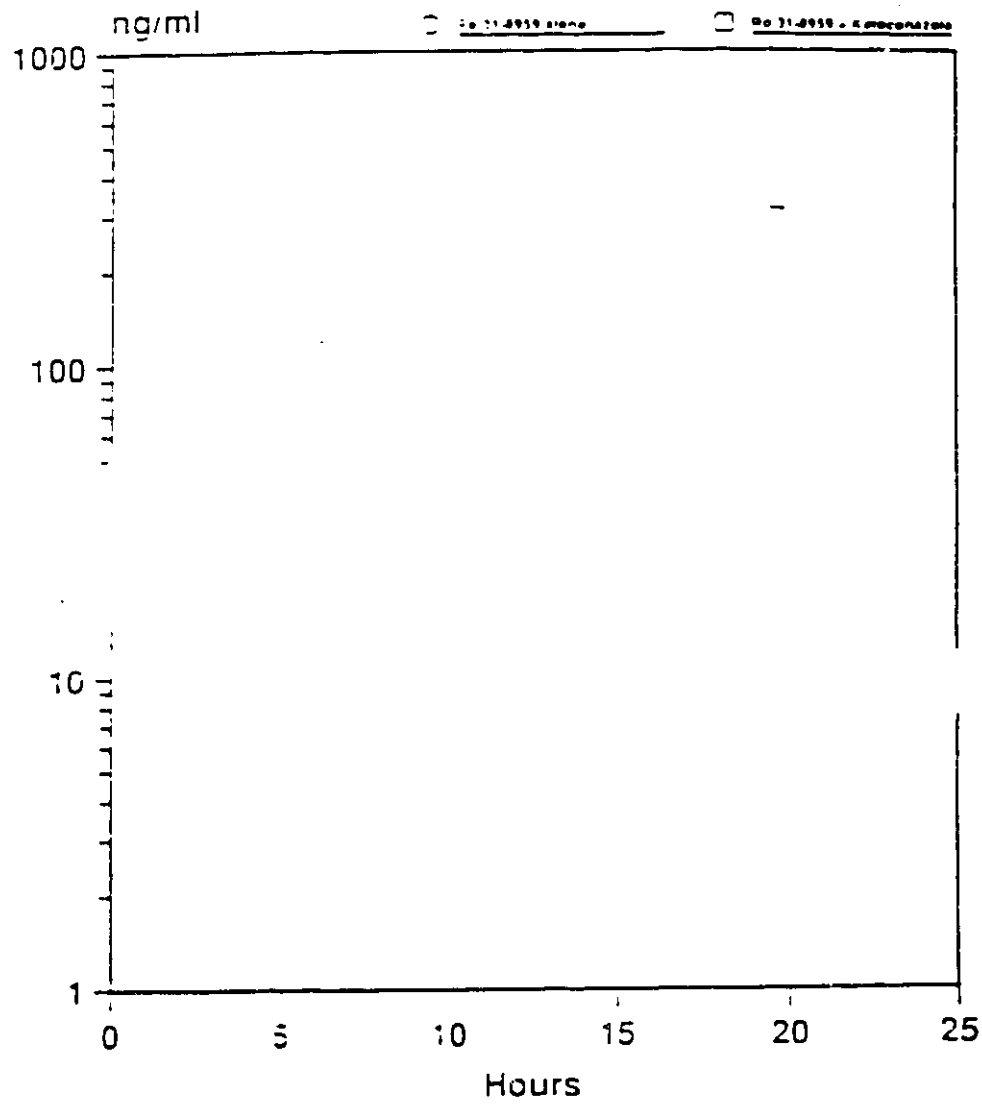


Figure 4

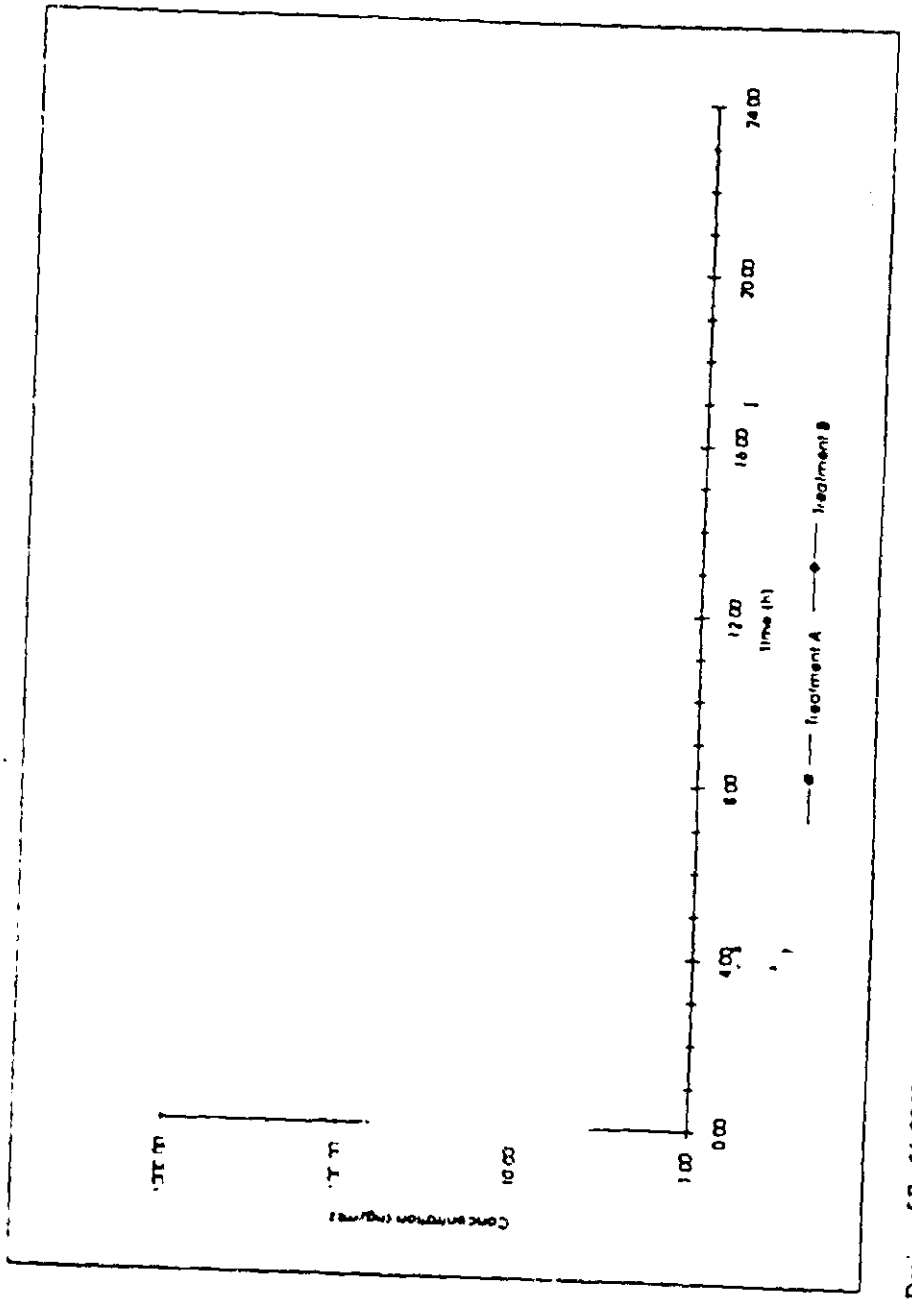


Figure 1 Mean Plasma Concentrations of Ro 31-8959 After Multiple Oral Dosing
Treatment A Ro 31 8959 600 mg t i d for 7 days
Treatment B Ro 31 8959 600 mg t i d plus rifampicin 600 mg once daily for 7 days

Dosing of Ro 31-8959 was at approximately 0, 6 and 12 h.

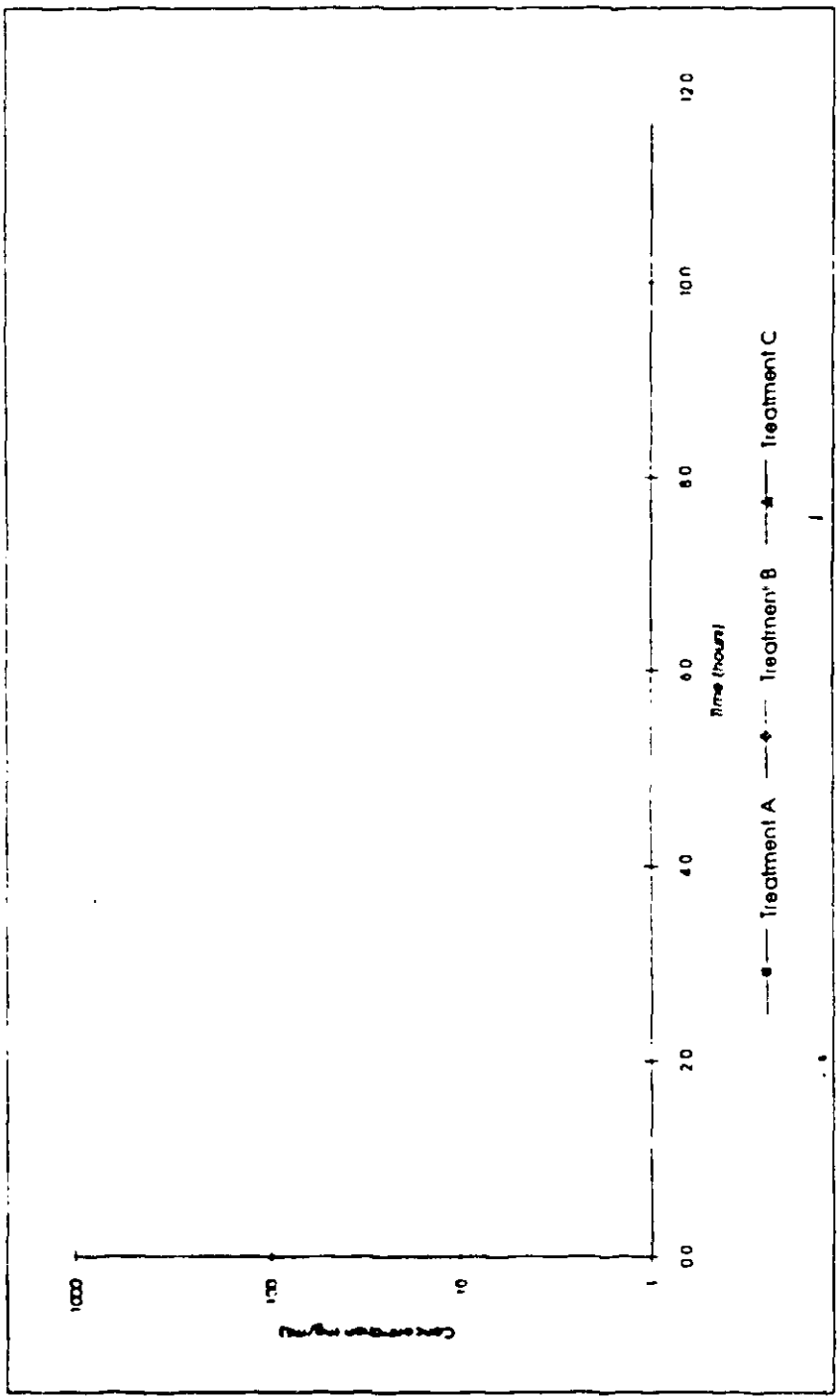


Figure 1. Mean Plasma Concentrations of Saquinavir (Ro 31-8959) After Single Oral Doses

Treatment A: 600 mg saquinavir given with water
Treatment B: 600 mg saquinavir given with double strength grapefruit juice
Treatment C: 600 mg saquinavir given with single strength grapefruit juice

Figure 2. Correlation Between $AUC_{(0-12)}$ Ratio and $AUC_{(0-12)}$ in Treatment A

Treatment A: 600 mg saquinavir given with water
Treatment B: 600 mg saquinavir given with double strength grapefruit juice
Treatment C: 600 mg saquinavir given with single strength grapefruit juice

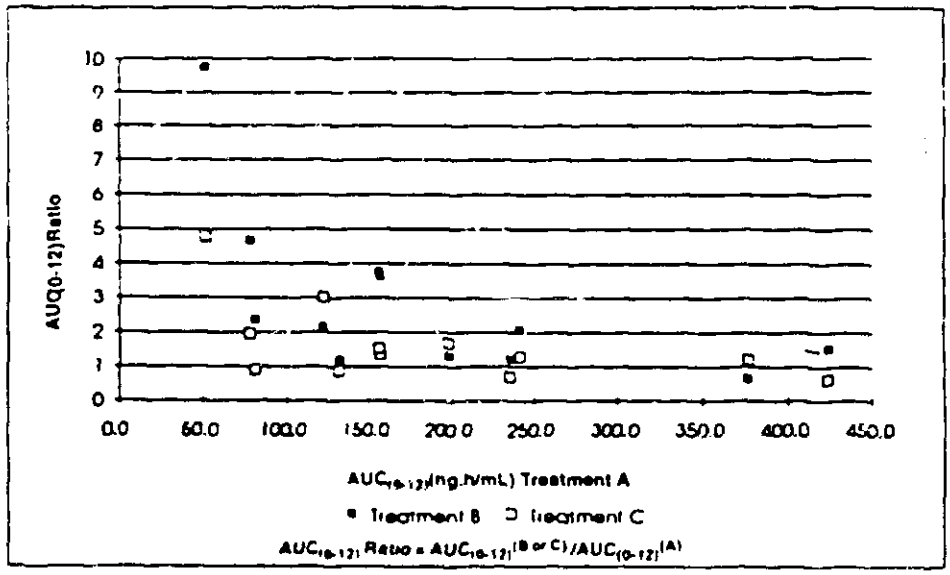


Figure 1 Mean Plasma concentrations of Ro 31-8959 During and After 4 Different Intravenous Infusions

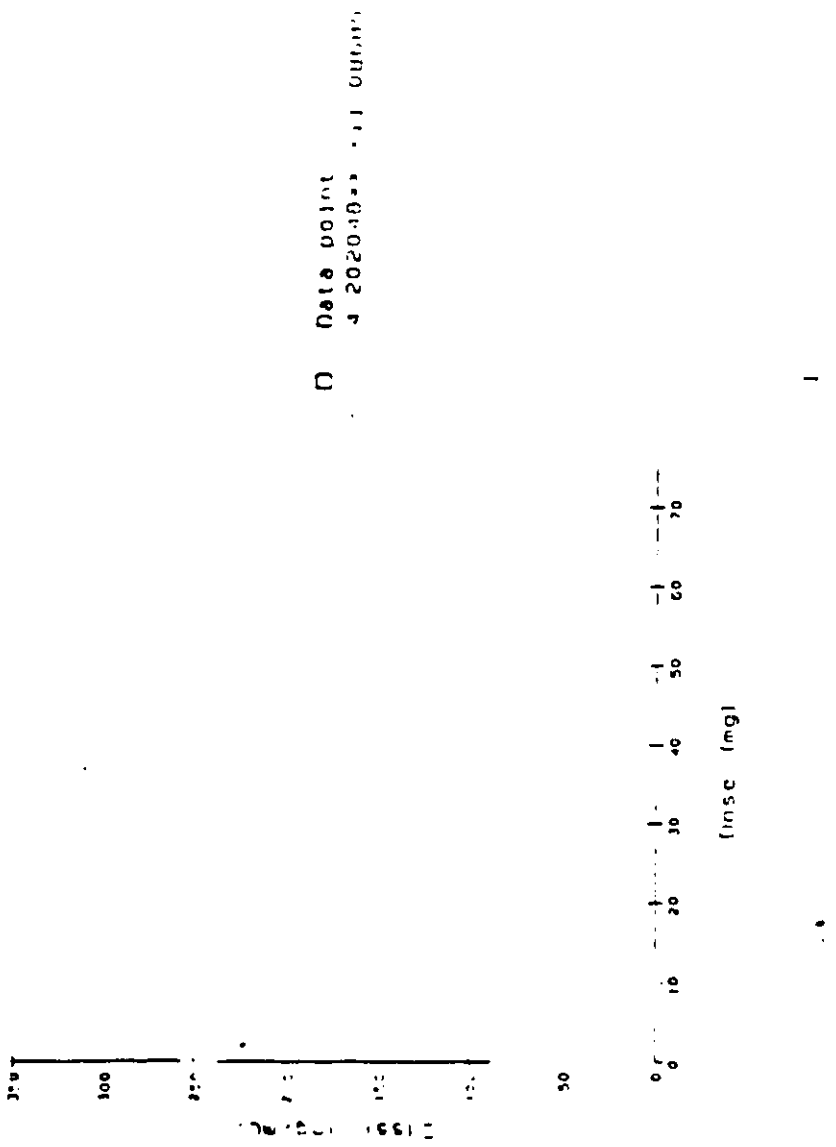
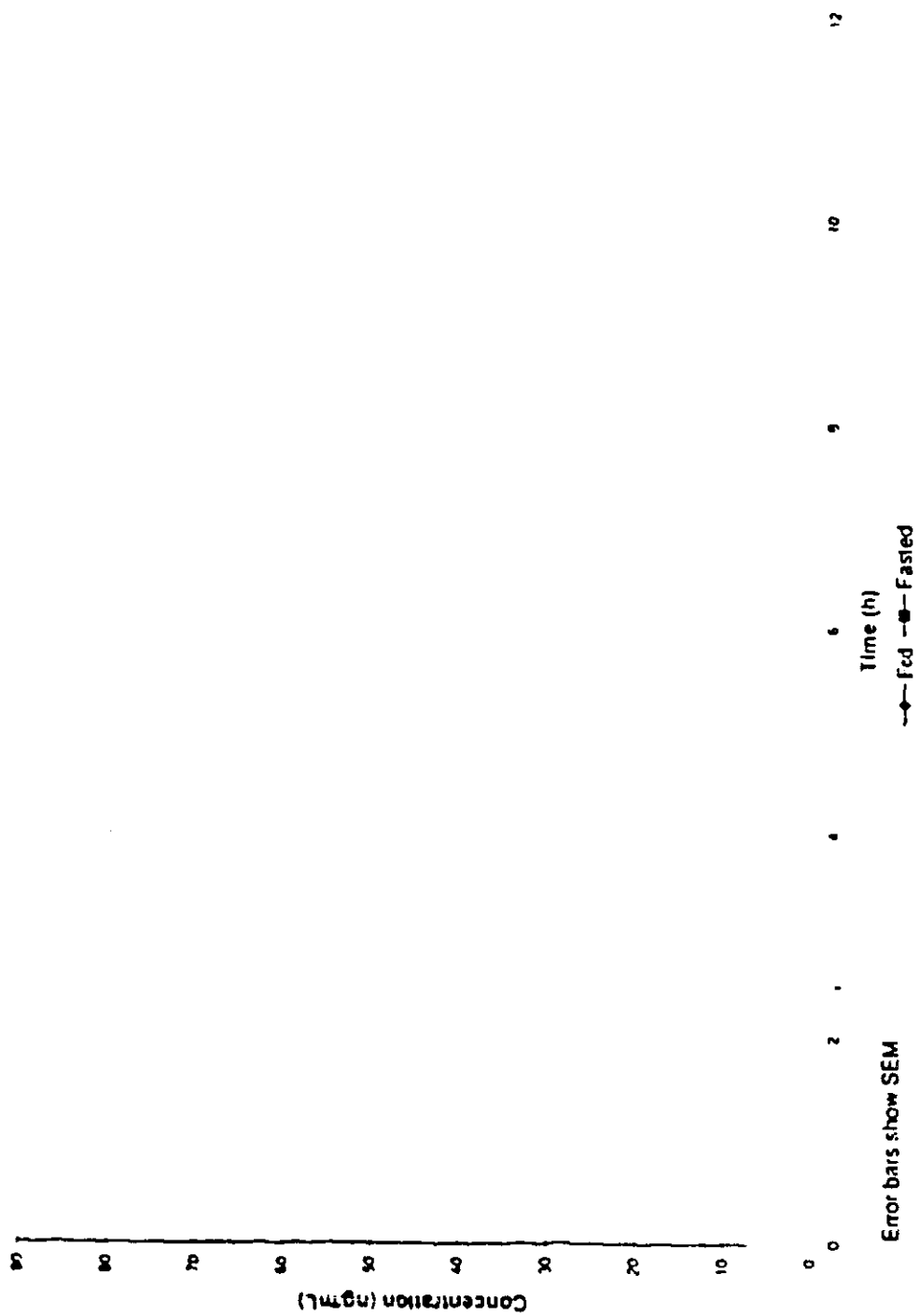


Figure 2 Individual Steady State Plasma Concentrations Versus Dosage After 3 Different Intravenous Infusion Rates (2, 12 and 24 mg/h)

9

Figure 1. Mean (n = 8) Plasma Concentrations of Saquinavir After Single Oral 600 mg Doses Given Fasted (Treatment B) or Fed (Treatment A)



Saquinavir Gastric Emptying and Lag Time

Study 144951

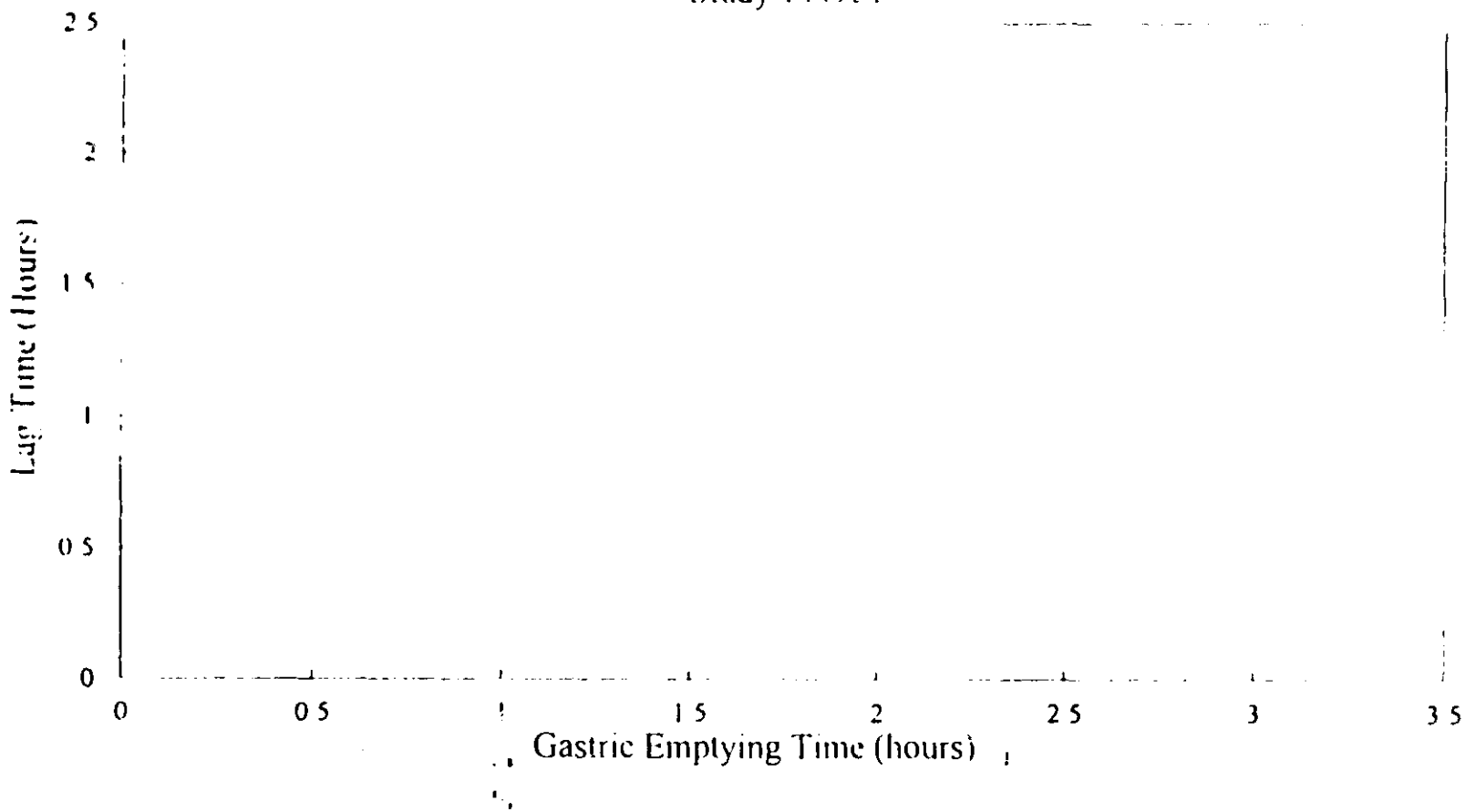


Figure 1 Mean plasma concentrations of Ro 31-8959 after single oral 600 mg doses

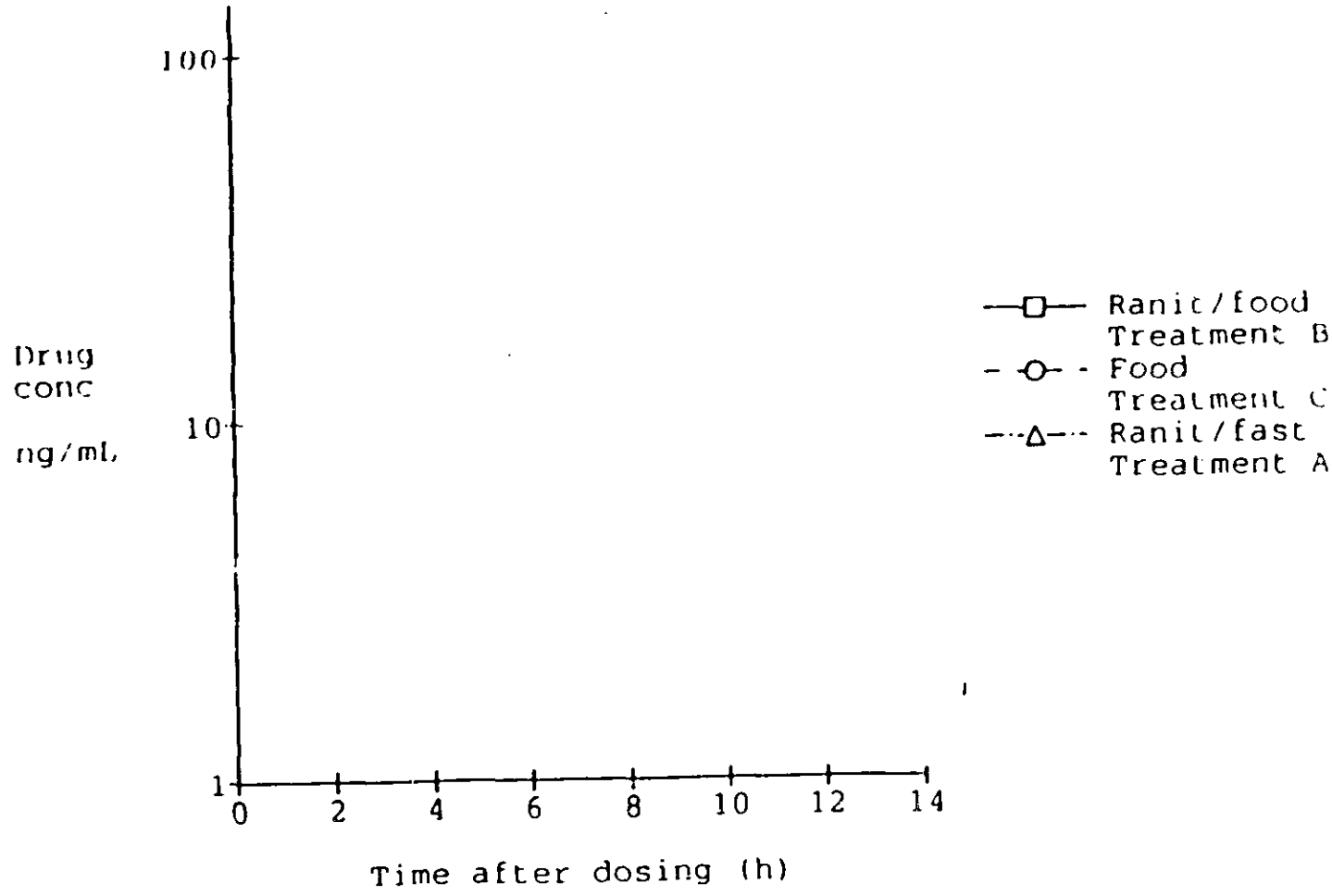


Figure 12

Figure 2 Mean Gastric pH (± SEM) Ranitidine without food (Treatment A)

T... 13

Figure 3 Mean Gastric pH (± SEM) Ranitidine with food (Treatment B)

Figure 14

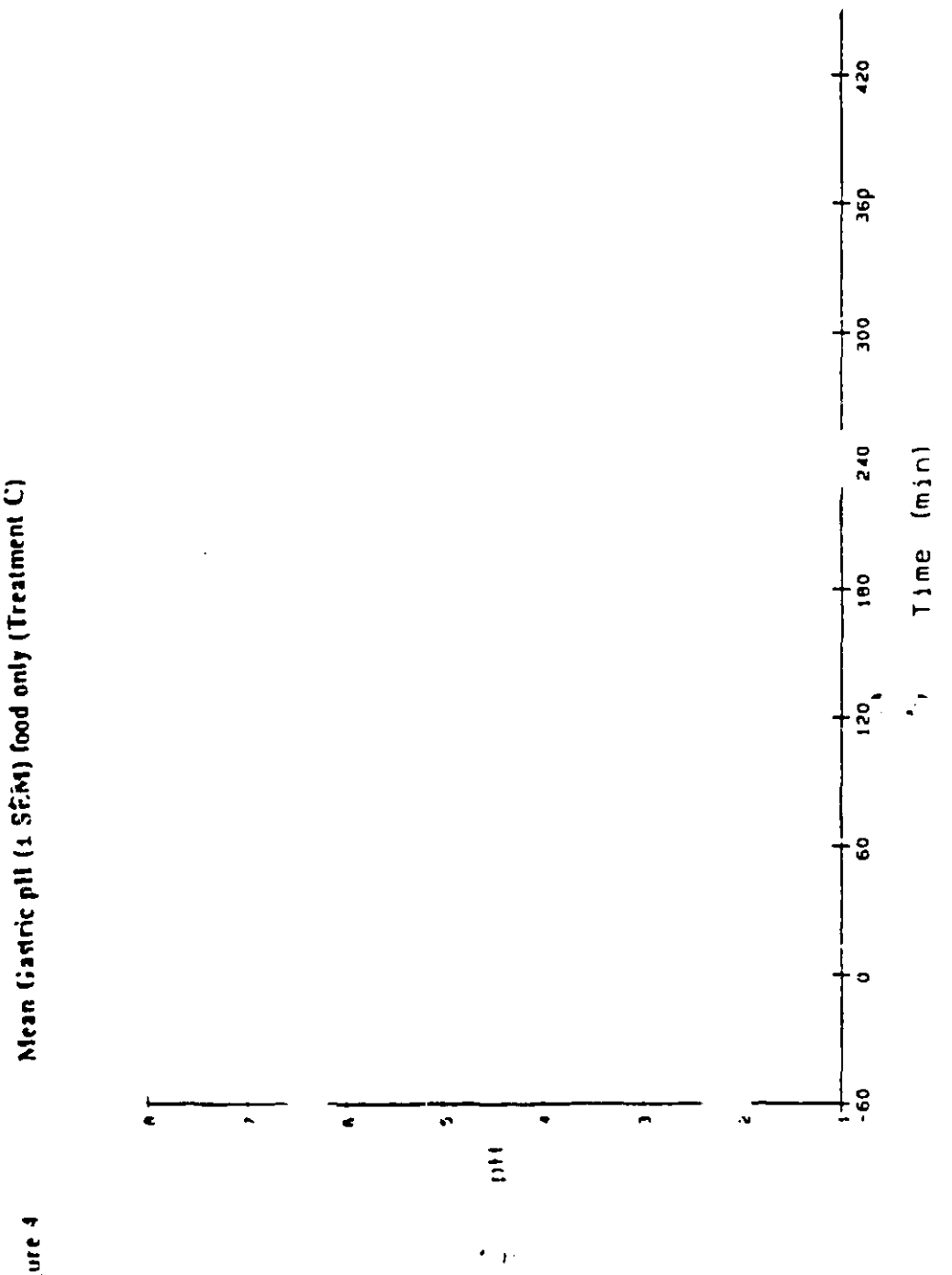
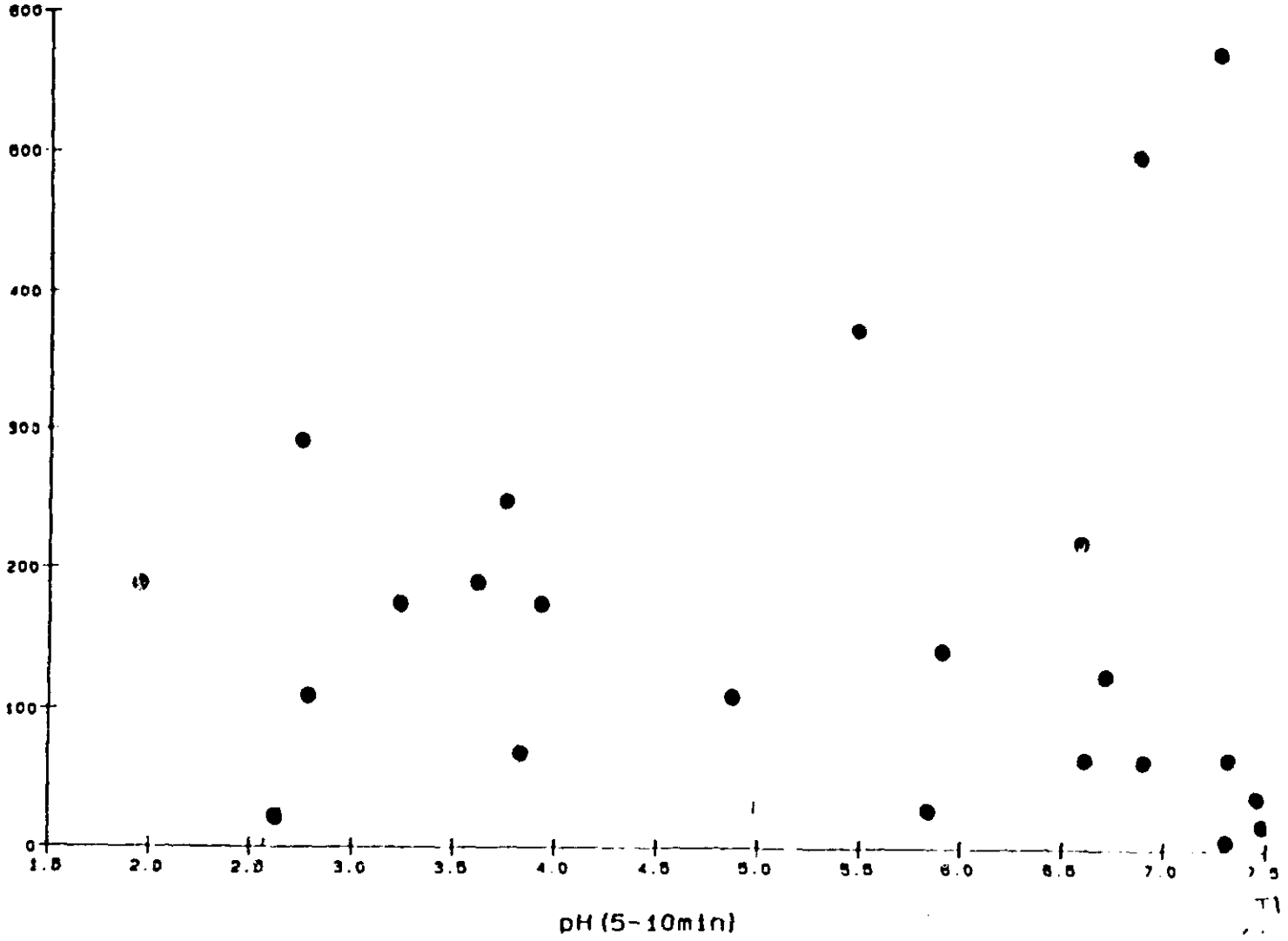
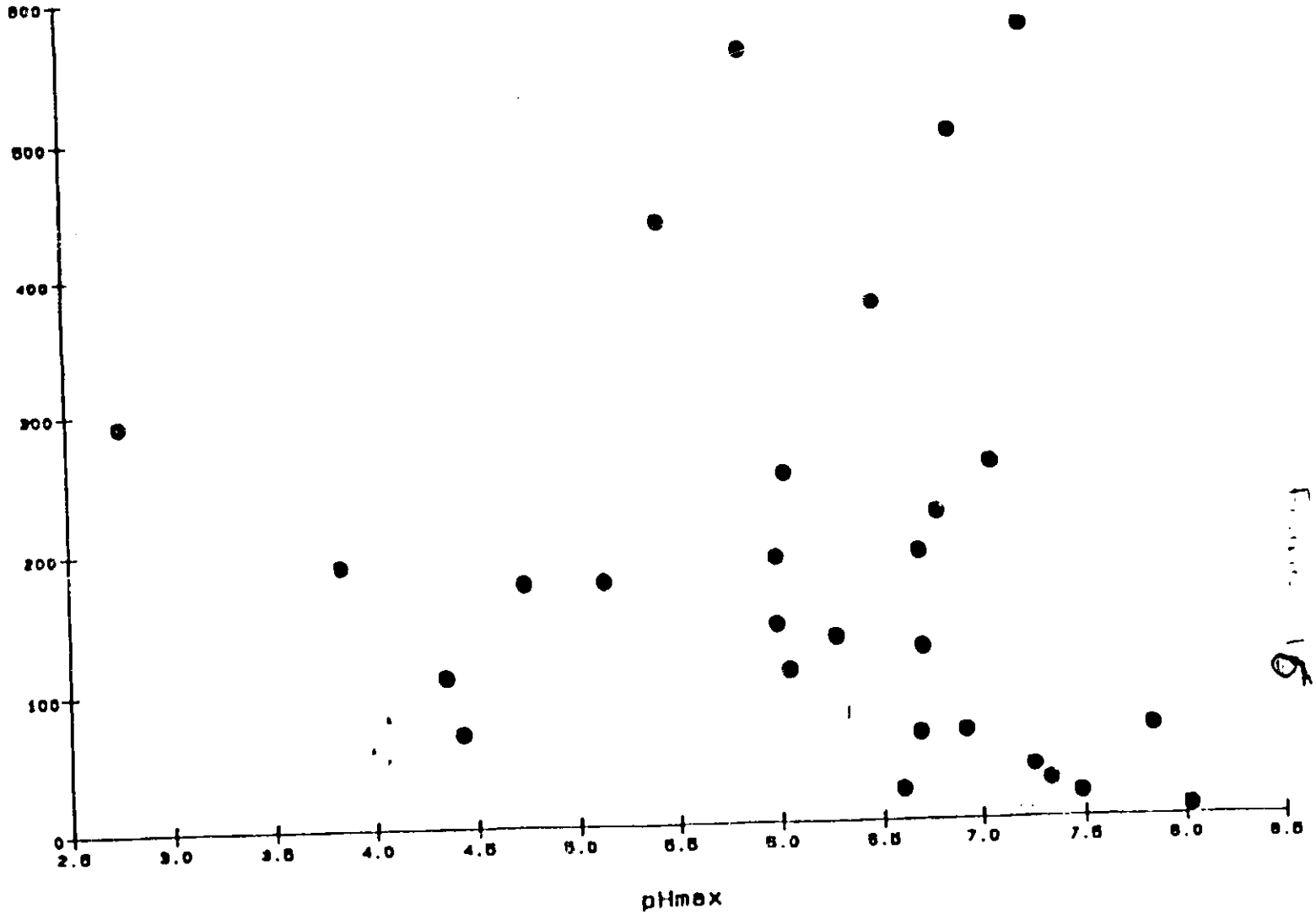

Mean Gastric pH (± SEM) food only (Treatment C)
pH
Time (min)
-60
0
60
120
180
240
300
360
420

Figure 5 Correlation of pH and Pharmacokinetic Parameters: pH5-10 versus Plasma AUC



ure 6

Correlation of pH and Pharmacokinetic Parameters: pHmax versus Plasma AUC



lasma AUC
ug.h/L)

WK14159A

32

W-142001

39

Figure 1 Mean plasma concentrations of Ro 31-8959 after three different timings of food intake relative to drug dosing

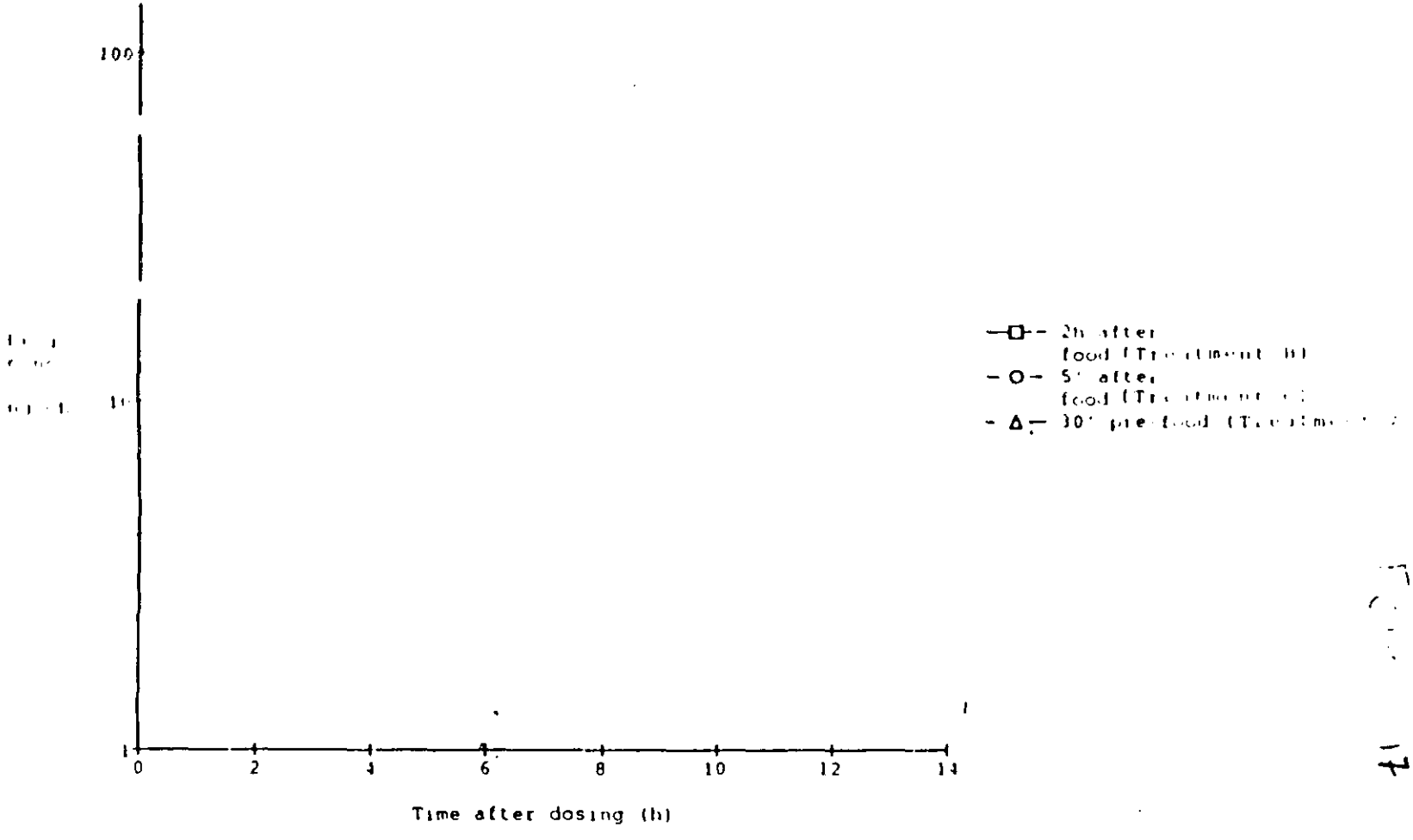
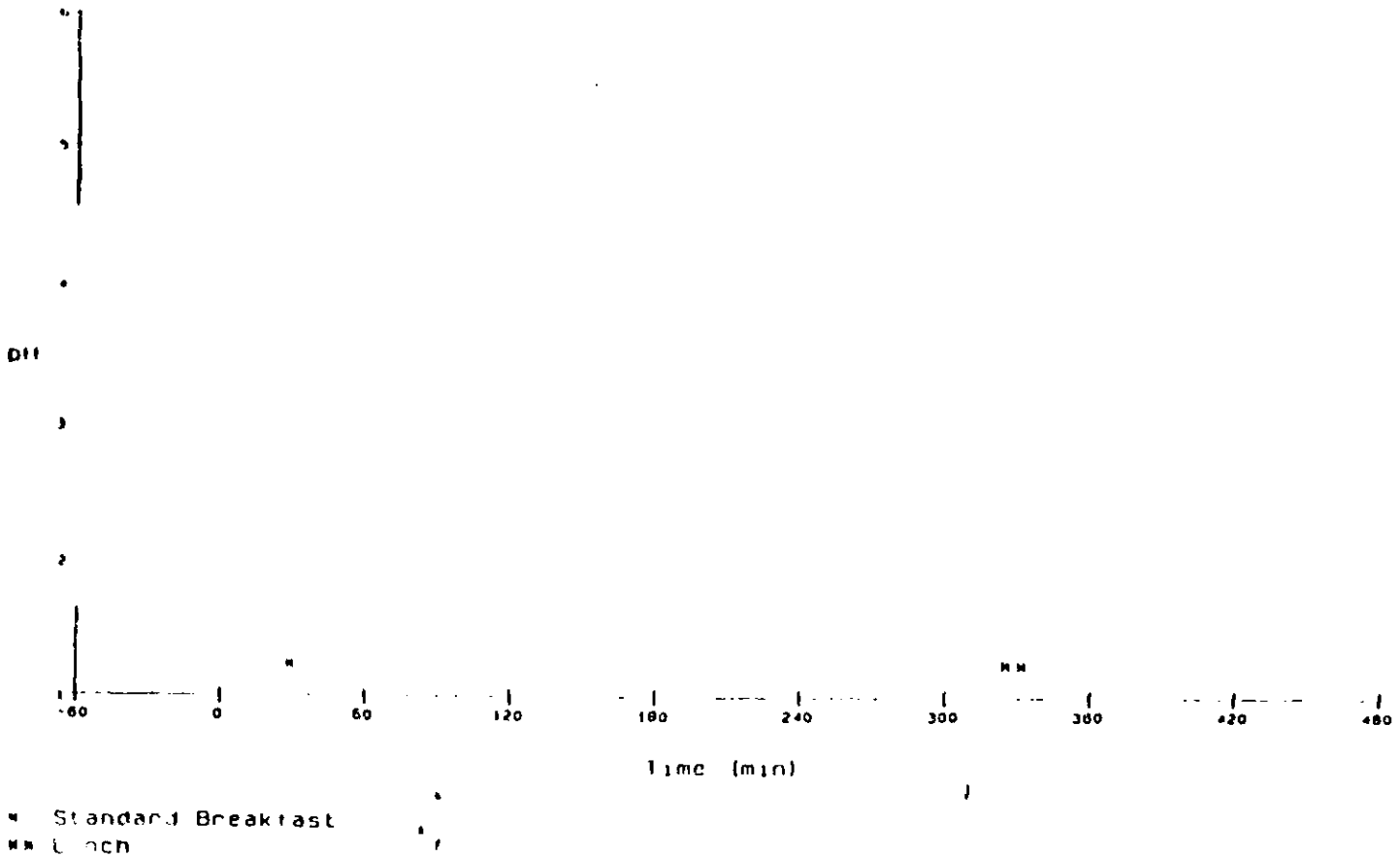


Figure 2 Mean Gastric pH (±SEM). Dosing 30 minutes before food (Treatment A)



WK14109A

WK14209

81

28

Figure 19

Figure 3 Mean Gastric pH (ESAI). Dosing 2 hours after food (Treatment B)

Figure 4 Mean Gastric pH (±SEM). Dosing 5 minutes after food (Treatment C)

20

Figure 21

Figure 5 Plot of pH and pharmacokinetic parameters (all subjects, all treatments): pHmax vs plasma AUC

Figure 22

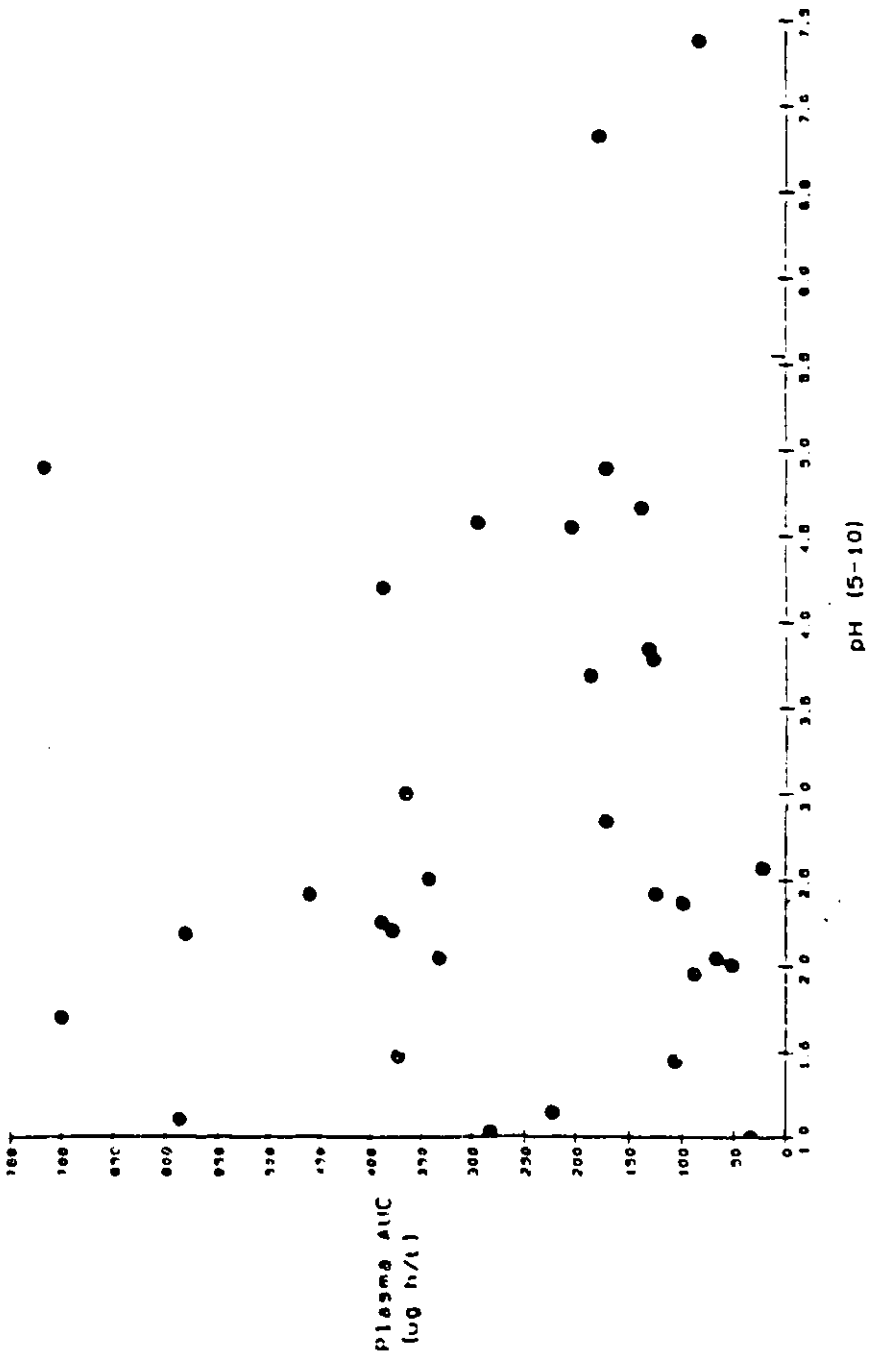


Figure 6 Plot of pH and pharmacokinetic parameters (all subjects, all treatments): pH5-10 vs plasma AUC

DIVISION OF ANTIVIRAL DRUG PRODUCTS

Review of Chemistry, Manufacturing, and Controls

1 ~~530~~

NDA #: 20-628

CHEMISTRY REVIEW #: 1 **DATE REVIEWED:** 08-Dec-95

SUBMISSION	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
Original	31-Aug-95	31-Aug-95	01-Sep-95
Amendment	04-Oct-95	13-Oct-95	13-Oct-95
Amendment	16-Oct-95	19-Oct-95	24-Oct-95
Amendment	23-Oct-95	25-Oct-95	31-Oct-95
Amendment	26-Oct-95	06-Nov-95	14-Nov-95
Amendment	20-Nov-95	22-Nov-95	22-Nov-95
Amendment	30-Nov-95	06-Dec-95	06-Dec-95
Amendment	05-Dec-95	07-Dec-95	07-Dec-95

NAME & ADDRESS OF SPONSOR Hoffmann-La Roche Pharmaceuticals Inc.
340 Kingsland Street
Nutley, NJ 07110

DRUG PRODUCT NAME

Proprietary:	INVIRASE®
Nonproprietary:	saquinavir mesylate
Code Name/#:	Ro 31-8959/000
Chem. Type/Ther. Class	1 P

PHARMACOLOGICAL CATEGORY Antiviral Anti-HIV

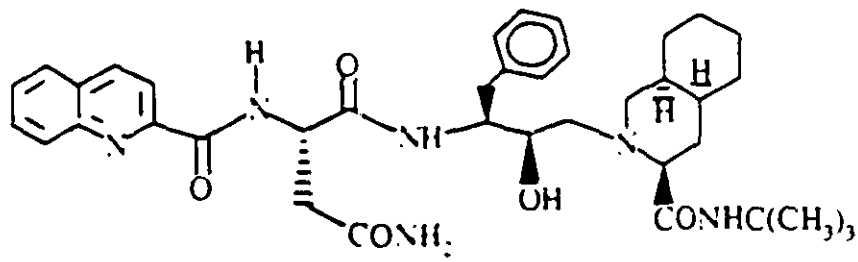
INDICATION Treatment of advanced HIV infection, in combination with a nucleoside analogue.

DOSAGE FORM/STRENGTH Capsules, 200 mg

ROUTE OF ADMINISTRATION Oral

CHEMICAL NAME/STRUCTURAL FORMULA

N-*tert*-Butyl-decahydro-2-[2(*R*)-hydroxy-4-phenyl-3(*S*)-[[*N*-(2-quinolylcarbonyl)-L-asparaginyl]amino]butyl]-(4a*S*, 8a*S*)-isoquinoline-3(*S*)-carboxamide methanesulfonate
($C_{38}H_{50}N_6O_5 \cdot CH_4O_3S$, Mol. Wt. 766.96)



$\cdot CH_3SO_3H$

Saquinavir mesylate

SUPPORTING DOCUMENTS

RELATED DOCUMENTS

Chemistry Reviews of IND
Record of facsimile of 28-Nov-95 comments and requests regarding drug substance & drug product)
Record of teleconference on 29-Sep-95

Record of teleconference on 06-Dec-95 (Discussion of 'Dissolution' specification)

CONSULT REVIEWS

Review of Tradenames (CDER Labeling and Nomenclature Committee, Consult # 471).
Environmental Assessment (N Sager, HFD-005)

CONCLUSIONS & RECOMMENDATIONS

The NDA submission and accompanying amendments provided adequate information on the chemistry, manufacturing and controls for INVIRASE The related cGMP and product specific inspections of the manufacturing facilities have been completed and are satisfactory. The Environmental Impact analysis is also acceptable The NDA, as amended, is therefore approvable from a chemistry standpoint

Paul S Liu, Review Chemist

Concurrence
HFD-530/Div Director
HFD-530/CChen 1/5/96

cc
Orig NDA 20-628
HFD-530/Div File
HFD-530/Div Director
HFD-530/CChen
HFD-830/ESheinin
HFD-530/PLiu
HFD-530/JMurray
HFD-530/KWu
HFD-530/NBansal
HFD-530/Jenkins
HFD-530/VKinsey
File: N-20628.000

OCT 30 1995

PHARMACOLOGIST REVIEW OF GLP EIR
(CP 7348.808)

FIRM NAME: F. Hoffmann-La Roche Ltd

CITY, STATE: Basel, Switzerland

CFN: 9692013

DISTRICT OFFICE: ORO

QUARTER/FISCAL YEAR ASSIGNED: 3/94

EI DATE(S): 04/18-20/95

INVESTIGATOR(S):
1. Jürg P. Seiler, IKS
2. Ruth Kaderli, IKS
3. Francesca Guiliani, IKS
4. Aurelia Oberli, IKS
5. David L. Duncan, HFC-134
6. Charles A. Snipes, HFD-345

INSPECTION TYPE:	____ ROUTINE SURV.	_X_ DIRECTED M.O.U.
FDA-483 ISSUED:	_X_ NO	____ YES
LETTER TO ISSUE:	_X_ NONE	____ PI LETTER
	____ WARNING LETTER	____ REJECTION OF STUDY

DATE EIR REC'D DSI: 8/15/95

DATE EIR REC'D BY REVIEWER: 8/15/95

DATE REVIEW COMPLETED: 10/13/95

FINAL EQ CLASSIFICATION: VAI

This facility is controlled by Roche Holding Ltd, a Swiss corporation. Roche Holding Ltd controls numerous other firms, among them this firm in Switzerland and (in the United States) Hoffmann-La Roche Inc., Genetech, Inc., and Syntex.

In the present inspection, the following studies were audited:

1. Study # 092R91
   Report # B-153194
   Completed: 11/23/92
   Study Title: Embryotoxicity and Teratogenicity Study in Rats with Oral (gavage) Administration of Ro 31-8959/008 (HIV Proteinase Inhibitor). Segment II Study without Postnatal Evaluation.
   Test Article: Ro 31-8959 (Invirase, Saquinavir)
   Type of Study: reproductive (Segment II)
   Testing Facility: this firm
   Sponsor: Hoffmann-La Roche Inc.
   Nutley, New Jersey
   IND:
   NDA: 20-628

2. Study # 021R92
   Report # B-154991
   Completed: 11/12/92
   Study Title: Embryotoxicity and Teratogenicity Study in the Rabbit with Oral (gavage) Administration of the HIV-Proteinase Inhibitor Ro 31-8959/613. Segment II Study.
   Test Article: Ro 31-8959 (Invirase, Saquinavir)
   Type of Study: reproductive (Segment II)
   Testing Facility: this firm
   Sponsor: Hoffmann-La Roche Inc.
   Nutley, New Jersey
   IND:
   NDA: 20-628

On 08/17/87 a CVM inspection at this facility resulted in a VAI-2 classification. In May of 1989, FDA Investigators Ernest Brisson and John Arnold participated in a joint inspection with Swiss authorities. The resulting classification was NAI. Subsequently, an inspection, classified NAI, was done 02/15/90 by the Swiss authorities alone under the memorandum of understanding with Switzerland.

The present inspection was conducted by the Swiss investigators, with the two FDA agents present as observers.

The EIR consists of a summary of findings, and reviews of the firm, its personnel, computer usage during the conduct of the two assigned studies (prepared by myself), the specific findings for audits of the two assigned studies, and nine points that were discussed extensively with management at the conclusion of the

inspection. To the EIR are appended six exhibits, including the 1993 annual report for the parent corporation, protocols and final reports (annotated by Investigator Duncan) for the two audited studies, a data file from one of the audited studies, a historical control reproductive toxicology data file, and an organization chart for the firm.

No Inspectional Observations Form FDA-483 was issued.

DISCUSSION:

I do not consider that the observations have fundamentally compromised the regulatory value of the studies. After returning from Switzerland, I telephoned Dr. K.-M. Wu, Pharmacologist in HFD-530, and conveyed this opinion.

The Swiss investigators and we had discussions with the facility's personnel about the following nine points, that are more fully described in the EIR itself:

1. Reporting by exception.
2. Incomplete reporting if findings are those that were expected.
3. A few minor transcription errors.
4. Lack of dosage preparation records for daily dilutions.
5. An additional statistical test was done without a protocol amendment.
6. Use of canned routine language in final reports when this language does not quite fit the circumstances.
7. No documentation of design level validation of the reliability of software.
8. No formal change control SOP for software and no documentation of QAU review of changes.
9. Incomplete training records.

On June 29, 1995, HFD-345 received a report signed by Dr. Ruth Kaderli and Dr. Jürg P. Seiler of the (Swiss) Intercantonal Office for the Control of Medicines, GLP Compliance Unit. This report, although prepared independently from the FDA's EIR, corresponds very closely to ours and reaches the same conclusions.

To forestall any confusion, I mention here that by the Swiss investigators' enumeration there were ten points, as they broke our point #8 down into two separate issues.

RECOMMENDATIONS:

1> Classify VAI

2> Thank the Swiss investigators for their courtesies during the inspection and acknowledge receipt of their inspection report.

Charles A. Snipes

Charles A. Snipes, Ph.D.
Pharmacologist

cc:
HFA-224
HFC-230/Woollen
HFD-340/RF/Lisook
HFC-133/Klug/Duncan
HFD-345/James/Fujiwara(3)/Snipes/CF
HFD-502
HFD-530/IND 41,099/NDA 20-628/Wu/Kinsey
DSI/NCLSB:Hoff-2.Rev
Draft: CAS: 10/13/95
Review: TKF/GWJ: 10/18/95
Finalize: CAS: 10/26/95
Format: kec: 10/26/95

Insp. Conc.: A
Dist. Dec. : E
HQ Class. : VAI

ENVIRONMENTAL ASSESSMENT

AND

FINDING OF NO SIGNIFICANT IMPACT

FOR

INVIRASE™

(saquinavir)

Capsules

NDA 20-628

FOOD AND DRUG ADMINISTRATION

CENTER FOR DRUG EVALUATION AND RESEARCH

DIVISION OF ANTIVIRAL DRUG PRODUCTS
(HFD-530)

FINDING OF NO SIGNIFICANT IMPACT

NDA 20-628

INVIRASE

(saquinavir)

Capsules

The National Environmental Policy Act of 1969 (NEPA) requires all Federal agencies to assess the environmental impact of their actions. FDA is required under NEPA to consider the environmental impact of approving certain drug product applications as an integral part of its regulatory process.

The Food and Drug Administration, Center for Drug Evaluation and Research has carefully considered the potential environmental impact of this action and has concluded that this action will not have a significant effect on the quality of the human environment and that an environmental impact statement therefore will not be prepared.

In support of their supplemental new drug application for INVIRASE, Hoffmann-La Roche Inc. has conducted a number of environmental studies and prepared an environmental assessment in accordance with 21 CFR 25.31a(a) (attached) which evaluates the potential environmental impacts of the manufacture, use and disposal of the product.

Saquinavir is a synthetic drug which is administered as an oral capsule in the treatment of Acquired Immunodeficiency Syndrome (AIDS) and AIDS-Related Complex (ARC). The drug substance will be manufactured at 12 different facilities identified in the environmental assessment. The drug product will be manufactured by F. Hoffmann-La Roche Ltd, Basel Switzerland or Hoffmann-La Roche Inc., Nutley, NJ. The finished drug product will be used in hospitals, clinics and by patients in their homes.

Saquinavir may enter the environment from excretion by patients, from disposal of pharmaceutical waste or from emissions from manufacturing sites.

Chemical and physical test results indicate that the drug entering the environment may exist in the aquatic or terrestrial environments. No rapid environmental depletion mechanism has been identified, although the compound is expected to bind tightly to soils and sediments thus limiting its bioavailability to environmental organisms As saquinavir is expected to persist in the environment for some time, the toxicity of the material to organisms was characterized. Studies were conducted to assess

the acute toxicity to water fleas (*Daphnia magna*), rainbow trout (*Oncorhynchus Mykiss*), green algae (*S. capricornutum*), the subacute toxicity to nightcrawlers (*Lumbricus terrestris*) and the inhibitory effect on microbial growth and activated sludge respiration. These studies indicate that there are no expected adverse environmental effects at the expected environmental concentrations.

Disposal may result from production waste such as out of specification lots or unused or expired product and user disposal of empty or partly used product and packaging. Pharmaceutical waste will be disposed of by the manufacturer at a licensed landfill or incineration facility. At U.S. hospitals and clinics, empty or partially empty packages will be disposed according to hospital/clinic procedures. From home use, empty or partially empty containers will typically be disposed of by a community's solid waste management system which may include landfills, incineration and recycling, while minimal quantities of unused drug may be disposed of in the sewer system.

Precautions taken at the sites of manufacture of the bulk product and its final formulation are expected to minimize occupational exposures and environmental release. Governmental certifications of compliance with environmental laws and/or emission requirements have been provided for several of the manufacturing facilities.

The Center for Drug Evaluation and Research has concluded that the product can be manufactured, used and disposed of without any expected adverse environmental effects. Adverse effects are not anticipated upon endangered or threatened species or upon property listed in or eligible for listing in the National Register of Historic Places

10/2/95
DATE

PREPARED BY
Nancy B. Sager
Environmental Scientist
Center for Drug Evaluation and Research

10/4/95
DATE

CONCURRED
Roger L. Williams, M.D.
Center for Drug Evaluation and Research

Attachment: Environmental Assessment

ORIGINAL NEW DRUG APPLICATION

ENVIRONMENTAL ASSESSMENT

INVIRASE™ (SAQUINAVIR MESYLATE)

CAPSULES

DISCLOSABLE VERSION

1. DATE

May 24, 1995

REVISED DATE

September 7, 1995

2. NAME OF APPLICANT

Hoffmann-La Roche Inc.

3. ADDRESS

340 Kingsland Street
Nutley, New Jersey 07110

4. DESCRIPTION OF PROPOSED ACTION

Hoffmann-La Roche Inc. is filing a new drug application for Invirase capsules for use in humans for the treatment of Acquired Immunodeficiency Syndrome (AIDS) and AIDS-Related Complex (ARC) throughout the United States. Invirase (saquinavir) 200 mg hard gelatin capsules are packaged in high density polyethylene bottles (425cc), fitted with a plastic safety cap (45mm) over a metal screw cap with pulp/polyethylene liner and glassine tacseal. Cotton used to protect the capsules. The capsule count per bottle is 270.

Saquinavir mesylate, the drug substance, is manufactured by F. Hoffmann-La Roche Ltd., Basel, Switzerland. The final dosage form may be produced at the F. Hoffmann-La Roche Ltd., Basle, Switzerland and/or at the Hoffmann-La Roche Inc. site in Nutley, New Jersey. The capsules will be packaged at the Hoffmann-La Roche Inc. facility in

be returned to Hoffmann-La Roche Inc. in Nutley, New Jersey for disposal in a lined industrial landfill or for incineration as described in Item 6.

The addresses for drug product manufacturing sites are as follows:

a. F. Hoffmann-La Roche Ltd
Grenzacherstr 124
CH-4002 Basel, Switzerland

b Hoffmann-La Roche Inc
340 Kingsland Street
Nutley, N.J. 07110

Manufacturing process of Ro 31-8959/008 is complex, multistep process. It is divided into 3 parts for synthesis of two key intermediates, Ro 47-0950 (= "Nisylate"), Ro 31-9373 (= "Decahydroamide") and Saquinavir mesylate. (Ro 31-8959/008). The synthesis flow chart is included in Appendix A (confidential). The following manufacturers will manufacture key intermediates.

Manufacturers of Key Intermediates

Part A: Ro 47-0950 (= "Nisylate")

Company	Address	Steps

Part B: Ro 31-9373 (= "Decahydroamide")

Company	Address	Steps

Part C: Ro 31-8959/008 (= Saquinavir mesylate[1])

Company	Address	Steps

The types of environments present adjacent to the production facilities are described below by site:

F. Hoffmann-La Roche Ltd., Basle, Switzerland

The Roche Basle plant is located on Basle city ground in a mixed industrial and residential zone at the Rhine river. The Basle plant occupies approximately 120,000 square meters area and is mostly covered with buildings. In the close proximity, the Ciba-Geigy and Sandoz plants are located northwest of the Roche plant. The Roche Basle plant is a manufacturing site for pharmaceuticals and chemicals for the Roche group. It is also a research and administrative site (Corporate headquarters) for the Roche group.

Hoffmann- La Roche Inc., Nutley, New Jersey

The Hoffmann-La Roche Inc. Plant is located approximately 10 miles west of the New York City in Nutley, New Jersey. The Nutley plant is located in an industrial/residential area. The state highway 3 runs along the north boundary of the site. The Passaic river is located approximately one mile east of the plant. The Nutley plant occupies approximately 122 acres of land and mostly occupied by office and manufacturing buildings. The entire state of New Jersey is a non-attainment zone for ozone, the Nutley environs are in attainment for all other criteria pollutants. The Roche Nutley plant is a manufacturing site for pharmaceuticals and other chemicals for the Roche group. It is also a research and an administrative site (US headquarters) for the Roche group.

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5. IDENTIFICATION OF CHEMICAL SUBSTANCES THAT ARE THE SUBJECT OF THE PROPOSED ACTION:

Proprietary Name: Invirase

Generic Name: Saquinavir mesylate (Ro 31-8959/008)

Chemical Name: cis-N-tert-Butyl-decahydro-2-[2(R)-hydroxy-4-phenyl-3(S)-[[N-2-quinolylcarbonyl)-L-asparaginyl]amino]butyl]-(4aS,8aS)-isoquinoline-3(S)-carboxamide methylsulfonate

CAS No: Not available

Molecular Formula: $C_{38}H_{50}N_6O_5 1:1 CH_4O_3S$

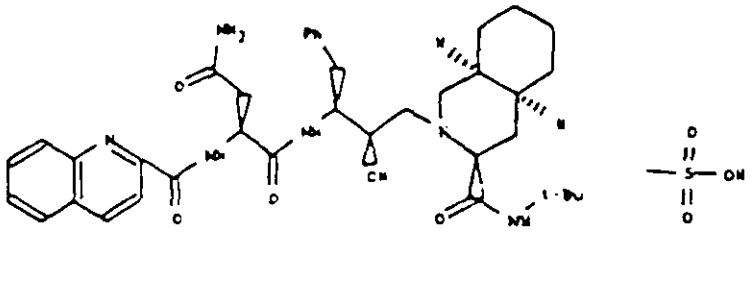
Molecular Weight: 766.9
670.8 Free Base

Melting Point: 245-249°C

Physical Description: White Crystalline Solid

Smile Notation:
CC(C)(C)NC(=O)C2CC(CCCC1C(NH+)2CC(O)C(Cc3ccccc3)NC(=O)NC(=O)c5ccc4ccccc4n5.CS(=O)(=O)=O
This is the SMILES for the salt structure, assuming that the isoquinoline nitrogen is protonated.

Structural Formula:



Saquinavir mesylate contains a maximum of 0.5% total of all impurities. The identified organic impurities are as follows

- Ro 31-9533 (Stereoisomer)
- Ro 61-4520 (By-product)
- Ro 31-9532 (By-product)
- Ro 31-9232 (aminoalcohol, precursor)
- Ro 31-9258 (S-quinargine, precursor)

These identified organic impurities consists of maximum of 0.1% each impurity in the final product. There are other individual impurities that consist of maximum of 0.05% each and total of 0.2% maximum impurities in the final product.

Since these impurities are in small amounts (within 0.5% total), the environmental concentrations are not likely to reach significant levels to cause any detrimental effects in the environment.

The physical chemical property data of Invirase and the Material Safety Data Sheet (MSDS) for Saquinavir mesylate is provided in the Appendix

B. The list of chemical substances associated with the manufacture of drug substance along with CAS No and copies available MSDSs is provided in confidential Appendix C The list of chemical substances associated with the manufacture of drug product along with CAS No. and copies available MSDSs is provided in confidential Appendix D.

6. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT:

The maximum projected annual use of Saquinavir mesylate for the five-year period following introduction of Invirase is provided in confidential Appendix E. All manufacturing operations are carried out under carefully controlled conditions and in compliance with applicable environmental regulations of the countries in which the operations occur. Compliance statements signed by a company official from various places are included in Appendix F. When available a certificate of compliance from appropriate foreign governmental authorities is included in Appendix G. Control of environmental emissions for the various manufacturing operations is outlined below.

6.1 Manufacture of Ro 47-0950 Vicenza, Italy

Ro 47-0950 is manufactured from the starting material 3-phenyl-1-alanine out at the FIS facility in Chemically Ro 47-0950 is p-nitrobenzene sulfonic acid(CAS 5S)-4-benzyl-2-oxo-5 oxalolidinyl) methyl ester.

is producing Ro 47-0950 under carefully controlled conditions and in compliance with relevant regulations, such as Ministerial Decree 203 of July 12, 1990. Applicable regulations are described in appropriate sections below.

6.1.1 Industrial Hygiene Controls

The manufacturing process is in accordance with the occupational law and the law for accident insurance. According to these regulations all laborers of the factory are subject to a periodical medical checkup.

Furthermore, the individual steps of the synthesis were subject to a careful risk analysis considering among other things the exposure and related health risks at the workplace Risk analysis is performed according to the following Decrees DPR n° 175 dd. May 17, 1988,

DPCM March 31, 1999, DL n° 626 dd. August 19, 1994 The required documentation has been duly supplied to the competent authorities.

Apart from that no specific measures have to be taken into account for the process in question if the usual individual rules on safety and hygiene are observed.

6.1.2 Substances Expected To Be Emitted - Manufacture of Ro 47-0950

During the manufacturing process some material may be released in the aqueous, terrestrial and air phases. The section below describes potential loss of material(s), containment devices utilized and their efficiencies, applicable permit information and other relevant regulatory information.

6.1.2.1 Air Emissions

Potential air emissions consist primarily of solvent vapors and fugitive dust. The waste gases of the manufacturing process are treated by primary measures such as filters with different specifications, condensers with cooling water and brine scrubbers or NaOH solution scrubbers.

The emitted gases correspond to the relevant European Economic Community (EEC) and Italian regulations. The chemicals listed in confidential Appendix C and D have the potential for released in this phase during the manufacturing of the Ro- 47-0950.

6 1.2.1.(a) Control Technology & Efficiency: Fugitive Dust

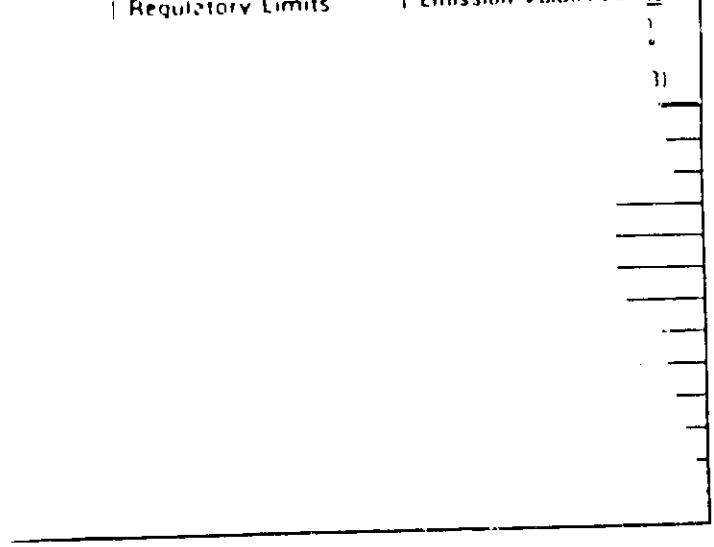
Local aspiration and dust collectors with greater than 98% efficiency. Both measures lead to a concentration of particulate matter less than 50 mg/m^3 at a mass flow of [illegible] kg/h or more as required by EEC and Italian air control regulations (President Decree DPR n 203 dd May 24, 1988)

6.1.2.1.(b) Control Technology and Efficiency: Organic Volatile Substances

Two bed active carbon adsorption system is utilized to contained volatile organic substances.

The Volatile organic Substances are classified according to the Ministerial Decree n 51 dd July 12, 1990 where the regulatory limits are indicated. FIS complies with the emission value limits prescribed by the Veneto Region Authorities in the Decree n°30 dd. Aug. 11, 1993. The following table shows the emission limits prescribed by various l regulatory authorities.

Emitted Substances	Classification	Regulatory Limits	Emission Values Limits



Regulations Governing Emissions

The relevant regulation is the ordinance to the protection of the air based on the President Decree n° 203 dd. May 24, 1988 relating to the protection of the environment. Allowable emissions of particulate matter and volatile organic substances from manufacturing processes are specified in the Ministerial Decree dd. July 12, 1990 which calls for the following limits:

Limit for Total Dust:

50 mg/m^3 at a mass flow of 0.5 kg/h or more,
150 mg/m^3 at a mass flow less than 0.5 kg/h.

Limit for Volatile Organic Substances

The listed volatile organic substances in the ordinance to the protection of the air are divided into 5 classes based on toxicological data and threshold limit values. The following limits are effective:

Class 1 5 mg/m^3 at a mass flow of 5 g/h
Class 2 20 mg/m^3 at a mass flow of 0.1 kg/h or more
Class 3 150 mg/m^3 at a mass flow of 2.0 kg/h or more
Class 4 300 mg/m^3 at a mass flow of 3.0 kg/h or more
Class 5 600 mg/m^3 at a mass flow of 4.0 kg/h

6.1.2.1. (c) Control of

The following control measures are utilized at the to reduced the air emission of

Regulatory Requirements of

Storage and use of phosgene is regulated in by the President Decree n. 175 dd May 17, 1988. According to the Art. 4, each manufacturer must send to the Environmental and Health Ministries in Rome a "notification", if he uses one or more dangerous substances and quantities used. The regulated quantity for phosgene is 750 kgs in Italy. Considering the quantity of phosgene that can be present at the plant (approximately 500 kgs) has provided to the Environmental Department of with a "declaration" reporting the risk analysis as required by the decree and other regulations.

Emission and Control of Phosgene at th Plant

The facility in uses diphosgene in the synthetic steps.

and Therefore, phosgene can be present as compound arising only by the decomposition of

The production and use of is carried out in a closed plant, which is maintained at reduced pressure with respect to the environment. A special control system has been installed at the FIS plant to reduces (eliminate) phosgene emissions. The environmental air changes are 10 cycles per hours. The air vent is equipped with scrubbers and NaOH scrubbers.

For the special phosgene emissions control system at the plant, please note the followings:

- The detector number 1, checks the efficiency of the process scrubber
- The detector number 2, checks the phosgene level in the air coming from the production building
- The detector number 3, checks the emission into the atmosphere. If a significant levels are detected then the

emission is automatically diverted to the general emission treatment plant

These controls provide a good safety margin and reduces the emissions of possible in the environment At the plant, the content of the emission have been always found below the detection limit during the production of

6.1.2.2 Wastewater Emissions: Rp 47-0950

The wastewater of the manufacturing process consist mainly of inorganic salts, different organic by-products and solvents.

6.1.2.2(a) Control Technology

One step biological wastewater treatment plant with a biodegradation capacity of 90% respect to Chemical Oxygen Demand (COD).

FIS biological treatment plant is provided with a permit according to Decree n° 1315 dd. March 08, 1991 issued by Region Authorities.

The outlet of the biological treatment plant conforms to the limits stated in the Art. 3 of the permit issued to FIS by the Mayor of Montecchio Maggiore - VI for the discharge of it to the consortium wastewater treatment plant, and in general, its compliance to the 49 individual parameters regulated by the permit for the use of the official sewerage issued by the Municipality of Montecchio Maggiore -VI and is regularly checked by the local authorities. The COD value is generally between 200- 400mg/L. The outlet of the consortium wastewater treatment plant are in compliance with 51 individual parameters reported in the relevant regulation (DPR 319 dd. May 10, 1976).

It is regularly and systematically monitored by Veneto Region authorities. The treated clarified

effluent is then discharged into the Brendola river.

6.1.2.2(b) Regulations Concerning the Prevention of Water Pollution

The regulation in force is the ordinance for waste water discharge, which is based on President Decree n° 319 dd. May 10, 1976 and on President Decree n° 650 dd. Dec 24, 1979 relating to the water protection against pollution. The ordinance considers a total of 51 parameters (e.g. temperature, color, odor, total suspended substances, pH-value, O_2-content, inorganic substances, individual heavy metals, phosphorous-content, COD, etc.) for which the official limits have to be observed.

6.1.2.3 Solid/Liquid Waste

Solid/liquid waste from the Ro 47-095C, manufacturing process consist primarily of solvents which are partly recovered, some organic by-products and filter aids from purification steps. Solid wastes are burnt in an incinerator which can treat at 950°C about 500 kg/h of hazardous solid.

6.1.2.3(a) Control Technology

The main part of the solvents is recovered in FIS distillation units and reused in subsequent runs of the same step of the synthesis.

Residue, inseparable solvents and solid wastes are incinerated in owned two incinerators equipped with an electrostatic precipitator and a wet scrubber system. The flue gas emitted is in compliance with the relevant regulations, in particular with the Authorization n° 6720 dd Dec 11, 1990 issued to Region Authorities.

Dusts coming from the electrostatic precipitator and the melted salts residual of the burning are

disposed in a municipal authorized dump according to Italian regulations (Ministerial Decree n° 915 dd Sept 10, 1982)

Incinerator plants are approved by Region authorities with the Decree n° 6720 of Dec. 11, 1990.

FIS holds a permit for the acceptance and treatment of special wastes according to the Italian Ordinance on movements of special wastes. This permit is regularly inspected by the local authorities with respect to

- the total emissions involved in the special waste treatment;
- the technical equipment involved;
- auxiliary equipments;
- the professional competence of the collaborators.

One hundred percent of the total special wastes of plant are treated in their own plant.

All activities relating to the treatment of special wastes are in accordance with the corresponding regulations.

6.1.2.3.(b) Regulations for Disposal of Wastes

The following regulations are related to the disposal of wastes:

- Ordinance on movements of special wastes - President Decree n° 915 dd. Sept 10, 1982
- Ordinance on treatment of special wastes - Regional Law n° 33 dd. April 16, 1985
- Technical ordinance on wastes - Regional Decree n° 2145

The ordinance on movements of special wastes regulates the disposal, transport and taking over of special wastes including import, export and transit. The taking over and handling of special wastes are connected with a corresponding permit issued by the competent authority.

The ordinance contains a list of special wastes divided into 28 categories according to which the contractor for further treatment has to classify and declare his special wastes.

The technical ordinance on wastes contains regulations for the diminution, the treatment and the utilization of wastes. In particular, the mode of treatment of the individual type of wastes, is prescribed and the requirements for the working of incineration equipment and landfills are specified

6.2 Manufacture of Ro 47-0950

Ro 47-0950 is manufactured by az, nder a specific agreement with Roche.

6.2.1 Industrial Hygiene Controls

The manufacturing process of include several steps. Many substances are used for the individual reactions. In the plant, these substances are known and all necessary measures are applied so that all collaborators are protected from the risks inherent to their utilization.

Material Safety Data sheet for chemicals in the plant is available to all collaborators. This data sheet includes information about: specific risks - toxicological risks - protection measures - first aids measures etc. For the dangerous substances, special detection installations are permanently in service in the manufacturing plants. Moreover, during the handling of some of these substances, there is a double partition between the operator and the substance

The federal laws are scrupulously respected. Federal and cantonal authorities make periodic verifications concerning the application of these laws.

The apparatus used for these manufacturing processes are systematically controlled.

The collaborators are subject to a periodical medical checkup.

A training program is instituted and everyone has to attend the lectures about hygiene and security, substances manipulation, apparatus utilization and manufacturing processes.

The factory is in compliance with the principles of security and the health protection promulgated by the "Société Suisse des Industries Chimiques" and the company is affiliated with it.

uses engineering controls to reduce or eliminate chemical exposure in the workplace wherever such controls are technically and economically feasible. When engineering controls prove not to be feasible or provide insufficient protection, the use of personal protective equipment is required. Prior to the start up of new equipment and/or processes, acceptance tests are carried out.

Prior to production involving a new drug substance which could potentially expose employees to a chemical which has no established exposure limit, the potential hazard is evaluated by experienced scientists and the safety team. Safety measures to be taken are listed on an internal chemical substance data sheet.

6.2.2 Substances Expected to be Emitted - Manufacture of Ro 47-0950

During the manufacturing process some material may be released in the aqueous, terrestrial and air phase. The section below describes potential loss of material(s), containment devices utilized and their efficiencies and applicable permit information and other relevant regulatory information.

6.2.2.1 Air Emissions

Potential air emissions consist primarily of solvent vapors (volatile organic compound, VOC) and fugitive dust.

6.2.2.1(a) Control Technology and Efficiency: Fugitive Dust

Local aspirators and dust collectors are used. During the manufacture of Ro 47-0950, no dust have been found in air emissions at

6.2.2.1(b) Control Technology and Efficiency: Organic Volatile Substances:

The following volatile organic compounds (confidential) may have potential to be released during the manufacture of Ro 47-0950.

(confidential list provided to FDA in confidential EA volume)

Waste gases from the manufacturing process are collected in a reservoir where solvents are condensed in a cool finger at about -10°C. The condenser exhaust is then combined with the general building exhaust air and routed to absorption scrubbers containing acidic or basic solutions as appropriate. The scrubber exhausts are directed to a treatment unit, which are of two kinds on north side of factory adsorption on active carbon is used and catalytic incineration on the south side of the factory.

With these measures the regulatory limits of

20 mg/m3 for class 1
100 mg/m3 for class 2
150 mg/m3 for class 3

are generally met for the adsorption on active carbon. The emissions remain well below these limits for the catalytic incineration. Both

installations always emit less than 3 kg VOC/hour

On both purification plants, on line control units are installed which records daily levels of air quality. This data is sent monthly to the environmental protection service of Valais.

Emissions are regularly inspected and monitored by the local regulatory authorities.

6.2.2.1(c) Regulations Governing Emissions

The relevant regulation is the "federal ordinance to the protection of the air". For the manufacturing process for RO 47-0950 is regulated by the following regulations:

- emissions of particular matter
- emissions of volatile organic compounds

Limits for total dust

If the flow is over 0.5 kg/hour, the concentration limit is 50 mg/m^3.

Limits for volatile organic compounds

Solvent Class	Flow[kg/h]	concentration limit [mg/m^3]
1	≥ 0.1	20
2	≥ 2.0	100
3	≥ 3.0	150

The total concentration has to be under 150 mg/m^3 and limits for solvent of classes 1 and 2 have to be followed.

6.2.2.2 Wastewater Emissions:

The wastewater from Ro 47-0950 manufacturing process consist of different

organic byproducts, solvents and inorganic salts.

(confidential list provided to FDA in confidential EA volume)

6.2.2.2(a) Control Technology

Two steps biological wastewater treatment plant using the "deep shaft" technology. The rate of degradation is about 90-95% (calculated on Total Organic Carbon (TOC) removal).

Effluents flow to the municipal treatment plant for further degradation (regulations call for a 85% elimination).

Wastewater generated in each individual step of RO 47-0950 synthesis (and all others too) are first pre-treated (by extraction or solvent distillation in order to reduce the organic content). Wastewater is then collected in reservoirs or drums until it has been analyzed by the environmental laboratory and approved for discharge to the wastewater treatment plant.

A full assessment on the wastewater treatment plant, regrouping about 15 parameters is provided to the environmental protection service of Valais every month. Furthermore, the effluent is analyzed every three months by the official cantonal laboratory.

Daily, 20 parameters are measured on raw water entering the treatment plant and the effluent by the plant personnel.

6.2.2.2(b) Regulations Concerning the Prevention of Water Pollution

The relevant regulation is the "federal ordinance for wastewater discharge". The ordinance considers a total of 52 parameters (e.g. color, pH-values, individual heavy metals, TOC, etc.).

The ordinance calls for 95% degradation on TOC

6.2.2.3 Solid/Liquid Waste:

Solid wastes from the manufacturing process consist of synthesis by products and filter aid from purification steps. Liquid wastes consist of solvents. None of the special wastes produced is treated by

(confidential list provided to FDA in confidential EA volume)

6.2.2.3(a) Control Technology

Solid wastes are stored in a cool dry place in drums. Wastes are analyzed and sent to an incineration facility for destruction. Storage and transport take place according to the Swiss ordinance on movement of special wastes.

The first foreseen objective concerning the environment is reduction at the source. A large part of recycling consist of the distillation of organic solvents. The distillate is re-used by the factory. Most of the incinerated solvents consist of distillation residues. These solvents are first neutralized and then centrifuged before being sent to outside company for incineration. Storage and transport take place according to the Swiss ordinance on movements of special wastes. Only a very small amount of pure solvents are introduced in a fuel burner to produce steam.

6.2.2.3(b) Regulations for Disposal of Wastes

The relevant regulation is the "federal ordinance on movements of special wastes". This ordinance regulate the disposal, transport and taking over of special wastes including import, export and transit. The taking over and

handling of special wastes are regulated by the competent governmental authority.

6.3 MANUFACTURE OF RO 47-0950

Ro 47-0950 may also be manufactured at under a special agreement with Roche. A certificate of Compliance signed high ranking company official and governmental official indicating that the manufacture of Ro 47-0950 at the plant is in accordance to the current laws and regulations of the purposes of environmental protection, is included in Appendix F and G, respectively.

6.4 Manufacture of Ro 47-0950:

Ro 47-0950 from starting material is manufactured a plants located i

6.4.1 OSHA Regulated Compounds

The workplace safety of the employees in the plants is regulated by a number of laws and ordinances. Included among these are, for example, special plant instructions in accordance with the Ordinance on Dangerous Substances, the requirement of pre-disposal investigations, a special catalog of procedures for protective procedures, special regulations, e.g. for cancer-inducing substances. The MAK values (MAK = maximum work place concentration), that have to aintained, are also relevant. These are specified in TR 900 (Technical Regulation for Dangerous Substances) Special requirements are promulgated by Accident Prevention Regulations (UVV). UVV 113, for example, regulates working with cancer-inducing hazardous substances takes additional precautionary measures such as the development of interna guidelines for safe concentrations in the work place Each plant has a hazard defense plan and processes are checked from a technical safety point of view in accordance with the internal Manual "Units and Procedural Safety" (in German) with the objective of recognizing and alleviating weak points in the system

The Interference Ordinance (12th BImSchV) is in operation in regard to the safe working of the units. A plant-specific safety analysis is required for poisonous and extremely poisonous substances

6.4.2 Substances Expected to be Emitted

During the manufacturing process some material may be released in the aqueous, terrestrial and air phases. The section below describes potential loss of material(s), containment devices utilized and their efficiencies, applicable permit information and other relevant regulatory information.

6.4.2.1 Air Emissions

Potential air emissions consist primarily of solvents and dust particles.

The production plants in the are equipped with many different systems such as waste air washers, filters and thermal waste air purification units in order to minimize emissions. In general, the efficiency of these are very high. The organic ingredients (dust particles) are incinerated

In the plant, the waste air from the plants is led to a central waste air incineration unit. This is equipped with a quench (a cooling and washing installation) and wet washing in order to reduce the emissions. The emissions of many parameters, such as sulfur dioxide and NO_x for example, are regulated.

The emissions are generally regulated in accordance with the Federal Emission-Protection Law (BImSchG), by official permit of the individual units and in accordance with "TA-Luft", a technical guide to keeping the air clean. The air emission limit values have to be observed in accordance with "TA Luft": In Federal Republic of Germany, "TA Luft" regulates emissions into the atmosphere. In addition, specific plant approval procedures exist in accordance with BImSchG in which the

main components, or characteristic products are limited

Limits for Total Dust

50 mg/m^3 at a mass flow of 0.5 kg/h or more, and 150 mg/m^3 at a mass flow of 0.5 kg/h.

Organic substances

The following limits are effective:

Class 1, 20 mg/m^3 a mass flow of 0.1 kg/h;

Class 2, 100 mg/m^3 a mass flow of 2 kg/h,

Class 3, 150 mg/m^3 a mass flow of 3kg/h,

Class 4 is for vapor-like inorganic substances and gaseous inorganic substance For this class, individual substances are defined as a function of their mass flow.

6.4.2.1.(a) Control of Phosgene

The following control measures utilized at facility to control the emissions All stages involved in the synthesis steps for producing is carried out at plant following the transfer of the from plant.

Regulatory Requirements of Phosgene in Germany

The MAK value (maximum allowable concentration) for phosgene is the most important parameter with respect to occupational safety in the plant The MAK value for phosgene (CAS No 75-44-5) is 0 1 ppm (ml/m^3) or 0 4 mg/m^3.

According to the German "TA Luft" regulations, a maximum concentration of phosgene emission allowed by mass is 1 mg/m^3 The Bayer plant at

holds a permit (permit number 3612, issued on December 21, 1992) for phosgene emissions

Emission and Control of Phosgene at the Plant

No is emitted during the production of at plant. During the manufacture of the is discharged into a special unit for a complete elimination. The off-gas stream (i.e. emissions from the special unit) is fed to the central thermal incinerator at plant for incineration

levels in the plant is periodically monitored by the on site environmental protection staff to assure the compliance with the regulatory limits Special sensors are installed in the ventilation system of the plant and the various operation centers in order to detect possible phosgene emissions. Analytical devices for phosgene measurements are installed at various points in suction lines in the production area In addition all employees and visitors of the plant are required to wear indicator badge The detection limit of the badge is 2 ppm

6.4.2.2 Wastewater Emissions

Both plants are equipped with a biological sewage treatment unit. The wastewater discharges are regulated by the specific start-up permit which is issued by the Waste Water Administration. The regulation governing these discharges is the § 7 a of the Water Management Law (WHG) together with Annex 22

– sewage treatment unit is a multi-stage installation. It discharges into the Rhine which is regulated by official limits. These limits are measured routinely and documented. In total, 10 parameters are regulated including, among others, the CSB (the Chemical Oxygen Demand, or COD), the BSB_5(the Biological Oxygen Demand, BOD), heavy metals, biological parameters and inorganic nitrogen. The elimination of the CSB amounts to > 85%. Nitrification and denitrification of ammonium[-based] nitrogen take place in the system.

A so-called wastewater register has been compiled as required by the start-up permit. This wastewater register contains information such the wastewater parameters have been described for the plants and other relevant information on minimization efforts. The manufacture of the Nisylate intermediates has been included in the start-up permit.

The works operates a communal sewage treatment unit together with the Wupperverband [a local group] in which, among others, the City of has been integrated into this system. The sewage treatment unit consists of several stages, e.g neutralization, preliminary clarification and biological clarification using "tower biology" [a biological tower] and open activated sludge basins, etc. The clarifying sludge is either incinerated or stored in dump own by

Seventy two parameters are subject to monitoring by the Authorities within the framework of the start-up permit. Both the concentrations and the throughput levels within a defined period of time are regulated. In this connection, summation parameters are registered such as the CSB or even individual

substances such as for example, chlorobenzene. In addition to official monitoring, other parameters are subject to so-called self-monitoring. The emissions data are made available to the Authorities on demand. CSB elimination amounts to more than 80%.

In also, the plants are combined by the wastewater register within the framework of the start-up permit to give production units in which the wastewater situation and purification operations are defined. The emissions are reduced by numerous decentralized procedures such as distillation, extraction, adsorption, etc. The discharge data for the intermediate is included in the wastewater register.

6.4.2.2(a) Regulations for Water Contamination

In the Federal Republic, the requirements in terms of reduction procedures for the ingredients of wastewater are regulated by § 7 a of the Water Management Law (WHG). For dangerous substances, reduction procedures are required according to the current state of the art. All other substances are regulated by Annex 22 of the Outline Waste Water Management Regulation derived from the Waste Water Origin Ordinance. The special parameters below are designated for the plant:

CSB, AOX, ammonium[-based] nitrogen, total phosphorus, sulfate, heavy metals such as mercury and cadmium, individual components such as chlorobenzene, chloroform, benzene, carbon tetrachloride, nitrotoluene, naphthalene, toluene, the xylenes, etc. The elimination of the individual components amounts, in some cases, to more than 90%.

The Ordinance concerning Units in regard to working with Substances that endanger Water and concerning Specialist Plants (VAwS) is a set of regulations with a specific aim of controlling substances that might endanger water by

installing special devices in the unit to prevent leakage.

6.4.2.3 Solid/Liquid Waste

Solvents and solvents by-products are generated as waste materials during the manufacture of Ro 47-0950. The solvents by-products are disposed of via incineration.

The majority of the solvents are recovered by distillation in the plant. Waste materials and residues following distillation is incinerated in the incinerators own by These incineration units are equipped with expensive purification devices for the smoke gas such as a quench, wet washers and, if required, an electrostatic dust separation unit with condensation (EGR). Steam is recovered during the incineration processes.

6.4.2.3(a) Regulations for the Disposal of Waste Material

Prior to disposal, each new waste must be reported has established their own policies and procedure for releasing waste for disposal. Then it is released by the District Government (formerly, the President of the Administrative District). It is released externally following obtaining disposal certificate from the regulatory authorities. The transportation to the disposal site regulated under the waste material way-bill. The basis of waste disposal is the Law concerning the Avoidance and Disposal of Waste Materials (Waste Materials Law - AbfG). This, among other, things involves the necessity for approving waste disposal plants, the obligation to publicize relevant data and monitoring The requirements in regard to recycling and alternative disposal of waste materials that require monitoring are defined in accordance with the current state of the art in the 2nd General Administration Regulation relating to the Waste Materials Law TA Abfall [a technical guide to waste material].

The Waste Material Monitoring Ordinance and the Residual Materials Monitoring Ordinance also have to be followed.

The incineration units are governed by the BImSchg and the 17th BImSchV, the works own plants are governed by a dump decree.

6.5 MANUFACTURE OF RO 31-9373:

Ro 31-9373 is manufactured from Z-L-Phenylalanine via several synthetic steps. Chemically, Ro 31-9373 is (3S,4aS,8aS)-N-tert-butyldecahydro-3-1'soquinoline carboxamide (= "Decahydro-amide"). is one of the contract manufacturing site for Roche for synthesis of Ro 31-9373. A certificate of compliance signed by council of the Origgio, is attached in Appendix G.

6.6 MANUFACTURE OF RO 31-9373:

Ro 31-9373 is manufactured by from Ro 31-9439 (Procos only performs 2 steps of the Ro 31-9373 manufacture).

6.6.1 OSHA Regulated Compounds

The manufacturing process is in accordance with the Italian occupational law and the law for accident insurance. According to these regulations all the collaborators of the factory are subjected to a periodical medical check-up. The plants are periodically inspected by the authority responsible for the safety and by a medical doctor.

All the factory and the production processes which are performed therein has been subjected to a thoroughly risk analysis according to the DPR 175/88 and DPCM 31.3.89 which implement the EEC directive No. 82/501 in Italy.

An update of the risk analysis is repeated every three years. Substantially no relevant environmental potential risk could be indicated as a result of the risk analysis performed at the Procos production plant.

6.6.2 Substances Expected to be Emitted

During the manufacturing process some material may be released in the aqueous, terrestrial and air phase. The section below describes potential loss of material(s), containment devices utilized and their efficiencies and applicable permit and other relevant regulatory information.

6.6.2.1 Air Emissions

Potential air emissions consist primarily of solvent vapors and fugitive dust. The waste gases of the manufacturing process are treated by primary measures such as filters with different specification, condensers with cooling water or brine scrubbers. The emitted gases correspond to the relevant Italian regulations.

6.6.2.1(a) Control Technology and Efficiency: Fugitive Dust

Local aspiration and dust collectors are utilized. Both measures lead to a concentration of particulate matter less than 50 mg/m^3 at a mass flow of 0.5 kg/h or more as required by Italian air control regulations.

6.6.2.1(b) Control Technology and Efficiency: Volatile Organic Substances

Condensers (water and brine) and scrubbers are utilized. With these measures the regulatory limits of less than 300, 600 and 300 mg/m^3 for isopropanol, hexane and toluene, respectively, are achieved.

6.6.2.1(c) Regulations for Air Emissions

The relevant regulation is the DPR 203/1988 and the annexed D.M. 12. July. 1990 governing allowable emissions of particulate matter and volatile organic substances from manufacturing processes.

Specifically the following limits are called for:

Limit for total dust

- 50 mg/m^3 at a mass flow of 0.5 Kg/h or more
- 150 mg/m^3 at a mass flow equal or more than 0.1 Kg/h and less than 0.5 Kg/h

Limit for volatile organic substances

The listed volatile organic substances in the ordinance for the protection of the air are divided into 5 classes based on toxicological data and threshold limit values. The following limits are effective:

Class 1: 5 mg/m^3 at a mass flow of 25 g/h or more

Class 2: 20 mg/m^3 at a mass flow of 0.1 Kg/h or more

Class 3: 150 mg/m^3 at a mass flow of 2 Kg/h or more

Class 4: 300 mg/m^3 at a mass flow of 3 Kg/h or more

Class 5: 600 mg/m^3 at a mass flow of 4 g/h or more

As far as individual compounds are concerned hexane is in the class 3 while toluene and isopropanol are in the class 4.

6.6.2.2 Wastewater Discharges

No wastewaters treatment from the steps of the Ro 31-9373 manufacturing process are performed at Procos. For other productions and general purpose, Procos is equipped with a physico-chemical process for the treatment of wastewaters. The outlet of the plant is

regularly controlled by local authorities. The effluent is discharged to the local sewage network and subjected to a biological purification plant under the management of the local authorities.

Limited amounts of wastewaters with high COD or salts content are sent to authorized incineration plants and the dependence to out side incineration plants will be reduced after the new wastewater concentration plant become operational.

6.6.2.2(a) Regulations Concerning the Prevention of Water Pollution

The regulation in force is the ordinance for wastewater discharge law May 1976 No. 319. The ordinance considers more than 50 parameters (e.g. temperature, color, total suspended substances, pH value or content, inorganic substances, individual heavy metals, phosphorous and nitrogen content, COD etc.) for which the corresponding indicated limits have to be observed.

6.6.2.3 Solid/Liquid Waste:

All activities relating to the treatment of special wastes are in accordance with corresponding regulations.

Solid wastes from the Ro 31-9373 manufacturing process consist mainly of exhausted hydrogenation catalyst mixed with filter aids or filter paper. Solid waste is carefully recovered and sent to specialized firms for the recovery of the catalyst. Liquid wastes from the Ro 31-9373 manufacturing process consist primarily of solvents and some organic byproducts from purification steps which are partially recovered.

6.6.2.3(a) Control Technology

Primarily mixtures of confidential chemicals are generated during the manufacture of Ro 31-9373.

The majority of the solvents are recovered and recycled. Residues, inseparable solvents and solid wastes are sent to selected authorized external incineration plants or burnt in a stream generator incinerator own by the The plant has the permit for burning mixtures of non-halogenated recovered solvents.

6.6.2.3(b) Regulations for Disposal of Wastes

Two relevant regulations apply to the disposal of wastes, namely the laws No. 915/82 and 475/88 and the annexed decree 27 July 1994.

The ordinances regulate the disposal, transport and taking over of special wastes including import, export and transit.

All activities depend on corresponding permissions from the competent authority.

In particular the treatment and utilization of wastes and the requirements for the management of incineration equipment and landfills are specified.

6.6.2.3(c) Other Emissions or Polluting Agents

The tolerable level of noise emission is regulated by DPCM 01.03.1991 for external noise and by D L 277/1991 for the level of noise inside the working departments.

6.7 Manufacture of RO 31-9373:

Ro 31-9373 is manufactured at

A compliance certificate from the government authority is provided in Appendix G.

6.8 Manufacture of Ro 31-9440

Ro 31-9440, a precursor of Ro 31-9373 in manufacturing process of Saquinavir mesylate

6.8.1 OSHA - Regulated Compounds

The manufacturing process is in accordance with the French Occupational laws and the laws for accident prevention. According to these regulations all collaborators of the factory are subjected to periodical medical check-up. Furthermore, the individual steps of the synthesis were subjected to careful analysis (fault tree method) particularly those using the phosgene.

6.8.2 Substances Expected to be Emitted

During the manufacturing process some material may be released in the aqueous, terrestrial and air phase. The section below describes potential loss of material(s), containment devices utilized and their efficiencies and applicable permit information and other relevant regulatory information.

6.8.2.1 Air Emissions

Potential air emissions consist primarily of solvent vapors and fugitive dust. The waste gases of the manufacturing process are treated by primary measures such as filters condensers with cooling water or liquid nitrogen, and brine scrubbers. The emitted gases correspond to the relevant French regulations.

6.8.2.1(a) Control Technology and Efficiency: Fugitive Dust

Local aspiration and dust collectors are utilized. Both measures lead to a concentration of particulate matter less than 50 mg/m^3 at a mass flow of 1.5 kg/h or more as required by French air control regulation.

6.8.2.1(b) Control Technology and Efficiency: Volatile Organic Substances

Condensers and water glycol mixture -25°C are utilized. Liquid nitrogen (-70°C) is utilized for phosgene. With these measures the regulatory limits of:

1 mg/m^3 for phosgene;
20 mg/m^3 for chlorinated solvents amino and ethers;
50 mg/m^3 for hydrogen chloride;
150 mg/m^3 for other organics are in compliance.

6.8.2.1(c) Regulation Concerning the Prevention of Air Pollution

The relevant regulation is an ordinance dated 03/01/1993 and issued in the official journal of the French Republic on March 28th 1993 p. 5283 to 5301. This ordinance includes annexes governing allowable emissions of particulate matters and volatile organic substances from manufacturing processes. Specifically, the ordinance calls for the following limit:

Limit for Total Dust:

50 mg/m^3 at a mass flow of 1.0 kg or more (Art. 27-1)

Limit for Volatile Substances

Among the many volatile substances ruled by the ordinance only the following are relevant for Ro 31-9440

- the hydrogen chloride 50 mg/m^3 at a mass flow of 1 kg or more (Art 27-5)

organic compounds at a mass flow of 2 kg/h or more. 150 mg/m^3 (Art. 27-71)

- organic compounds listed in Annexe III at a mass flow of 0,1 kg/h or more 20 mg/m3 (Art 27.7.2) phosgene 1 mg/m3 at a mass flow of 10 g/h or more (Art 27 9 1)

6.8.2.2 Wastewater Discharges

The wastewater of the Ro 31-9440 manufacturing process consist mainly of inorganic salts, different organic by-products and solvents.

6.8.2.2(a) Control Technology and Efficiency

Only cooling water is discharged directly to the sewer. Wash water if checked as being non-polluting is also discharged to the sewer otherwise it is treated as process wastewater. The process wastewater handled by a specialized and officially approved company.

Process wastewater and chlorinated solvents are burned by a specialized and officially approved company. Other solvents are recycled after treatment by a specialized and officially approved company.

6.8.2.2(b) Regulations Concerning the Prevention of Water Pollution

The same ordinance deals with water pollution in the articles 31 to 35 and 60. It considers many parameters: mass flow, temperature, pH value, color suspended substances DOC, DOB, other substances and heavy metals. It also regulates wastewater treatment plants. The local authorities are allowed to reduce the limits according to specific local situations.

6.8.2.2(c) Regulations for Disposal of Wastes

The same ordinance also rules disposal of wastes (articles 44, 45, 46). It deals with rather general guide lines which are to be precise in the particular permits. The wastewater (cooling and plant washing), is constantly sampled and analyzed by equipment located on the sewer before disposal in the Juine river. The analytical results have to be submitted to the local authorities.

6.8.2.3 Solid/Liquid Wastes

Solid/liquid wastes from Ro 31-9440 manufacturing process consist primarily of solvents which are recovered.

(confidential list provided to FDA in confidential EA volume)

The distillation is processed by Speichim a registered specialized and officially approved company. The burning is processed by GEREP another registered specialized and officially approved company. Companies are allowed to burn or to proceed to special disposal of wastes are regulate by the law issued on July 15, 1975

6.9 Manufacture of Drug Substance-F. Hoffmann-La Roche Ltd. Basle, Switzerland

The drug substance is synthesized from Ro 31-9373 at the F. Hoffmann-la Roche Ltd facility in Basle, Switzerland. Saquinavir mesylate is manufactured in intermediate scale general purpose production equipment. All manufacturing operations carried out at the F. Hoffmann-La Roche Ltd facility in Basle, Switzerland, are under carefully controlled conditions and in compliance with the rules of Good Manufacturing Practices and the Swiss legislation. Applicable laws include

- Federal law relating to the protection of the environmental SR 814.01 (October 7, 1983) (Umweltschutzgesetz).

- Federal law relating to the water protection against pollution SR 814 20 (October 8, 19710) (Gewasserschutzgesetz).

- Ordinance to the protection of the air SR 814.318.142 (December 16, 1985) (Luftreinhalteverordnung)

- Ordinance relating to environmentally hazardous substances SR 814.013 (June 9, 1986) (Stoffverordnung).

- Ordinance for wastewater discharge SR 814.225.21 (December 8, 1975) (Verordnung uber Abwasserentsorgung)

- Ordinance relating to the assessment of the environmental impact SR 814.011 (October 19, 1988) (Verordnung Uber die Umweltvertraglichkeitsprufung).

- Ordinance relating to the protection against noise SR 814.41 (December 15, 1986) (Larmschutzverordnung).

- Ordinance on movements of special wastes SR 814.014 (November 12, 1986) (Verordnung uber den Verkehr mit Sonderabfallen).

- Technical ordinance on wastes SR 814.15 (December 10, 1990) (Technische Verordnung Uber Abfaulle).

Applicable provisions of these laws are described in the appropriate sections below

The manufacturing equipment of Roche Basle consists mainly of multipurpose units in which different products are manufactured on a routine basis For this reason, there exist no official permits for specific single manufacturing processes. On the contrary, the official permits are obtained for the individual equipment or possibly for a group of equipments

The procedure for obtaining a permit is initiated by an application for the construction of the unit addressed to the building inspectorate of the district (Canton) Basel-Stadt. After investigation of the application by the competent authorities (among others the department for water protection, the public health department, the department for protection of the air, the cantonal inspectorate for the fire-brigade, etc.) permission for construction is given under specific requirements which have to be fulfilled during construction and when the unit is operated.

Among other things an official permit is necessary for:

- new industrial equipment;
- enlargements of industrial units;
- replacement of production facilities;
- enlargement of production capacities if associated with an increase in the hazardous risk potential or with an increase in the pollution of the environment;
- new installation of specific apparatuses such as desiccators belt filters, etc.

The finished unit is inspected by the competent authorities and the company is provided with a permit for operating the equipment which is valid for the manufacturing of any product.

The following table is giving an overview on the equipment used in the manufacturing of Saquinavir mesylate, the location of the different multipurpose units as well as on the date of the permit applicable to the corresponding unit:

Building	Multipurpose Unit No.	Permit Dated
31	MZ 102	October 8, 1980
	MZ 108	October 8, 1980
	MZ 138	October 8, 1980
	MZ 152	March 20, 1974
	MZ 160	October 8, 1980
34	MZ 018	October 8, 1980
40	MZ 053	October 8, 1980
	MZ 096	October 21, 1991
43	MZ 007	January 10, 1994
	MZ 008	July 8, 1986
	MZ 010	October 21, 1991
	MZ 113	October 8, 1980
	MZ 116	June 12, 1979
	MZ 122	January 23, 1987
	MZ 123	July 8, 1986
	MZ 220	July 8, 1986
50	MZ 031	April 27, 1979

	MZ 044	April 16, 1993
	MZ 058	April 29, 1985
	MZ 234	December 15, 1989

Apart from the permits for the individual manufacturing units the company holds a permission for running the industrial wastewater treatment plant of Ciba-Geigy and Roche.

6.9.1 OSHA - Regulated Compounds

The chemical substances associated with the manufacture of Invirase are listed in Appendix C and D. Also included in Appendix C and D are copies of available Material Safety Data Sheets (MSDS). The list of chemical substances and the set of MSDSs are considered to be confidential in order to protect manufacturing process and not to be released by the Agency without the express permission of Hoffmann-La Roche Inc.

The manufacturing process is in accordance with the Swiss Federal Occupational Law and the law for Accident Insurance. According to these regulations all laborers of the factory are subject to a periodical medical checkup. Furthermore the individual steps of the synthesis were subject to a careful risk analysis considering among other things the exposure and related risks at the workplace. In addition, the Group Directive "Occupational Hygiene Directive" applies to the manufacturing process. In line with this directive handling of Invirase is performed in closed systems. Apart from that, no specific measures have to be taken into account for the process in question if the usual individual rules on safety and hygiene are observed.

6.9.2 Substances Expected to be Emitted

During the manufacturing process some material may be released in the aqueous, terrestrial and air phase. The section below describes potential loss of material(s), containment devices utilized and their efficiencies and applicable permit information and other relevant regulatory information.

6.9.2.1 Air Emissions

Potential air emissions consist primarily of solvent vapors and fugitive dust. The waste gases of the manufacturing process are treated by primary measures such as filters with different specifications, condensers with cooling water, and brine or ammonia scrubbers. The emitted gases correspond to the relevant Swiss regulations

6.9.2.1(a) Control Technology and Efficiency: Fugitive Dust

Various buildings mentioned in Table are equipped with local aspiration and dust collectors with greater than 98% efficiency. Both measures lead to a concentration of particulate matter less than 50 mg/m^3 at a mass flow of 0.5 kg/h or more as required by Swiss Air Control Regulations.

6.9.2.1(b) Control Technology and Efficiency: Volatile Organic Substances

Volatile organic substances such confidential list of chemicals are used in the manufacture of Saquinavir mesylate drug substance. In general, organic volatiles are passed through fume incineration with approximately 99.9% efficiency. With these control measures the regulatory limits of 20 mg/m^3 for class 1, 100 mg/m^3 for class 2 and 150 mg/m^3 for class 3 are well met.

The relevant regulation is the ordinance to the protection of the air based on the federal law relating to the protection of the environment. The regulations include annexes governing allowable emissions of particulate matter and volatile organic substances from manufacturing processes. Specifically, the ordinance calls for the following limits:

Limit for Total Dust:

50 mg/m3 at a mass flow of 0.5 kg/h or more.

Limits for Volatile Organic Substances

The listed volatile organic substances in the ordinance to the protection of the air are divided into 3 classes based on toxicological data and threshold limit values. The following limits are effective:

Class 1: 20 mg/m3 at a mass flow of 0.1 kg/h or more

Class 2: 100 mg/m3 at a mass flow of 2.0 kg/h or more

Class 3: 150 mg/m3 at a mass flow of 3.0 kg/h or more

Those volatile organic substances which are not classified, are treated according to the ordinance and are classified by an internal standard procedure based on toxicological data and threshold limit values.

The future manufacture of Saquinavir mesylate will be in compliance with provisions of this permit or similar permits for alternative equipment.

6.9.2.2 Wastewater Discharges

The wastewaters from the Invirase manufacturing process consist mainly of inorganic salts, different organic byproducts and solvents. During the manufacturing, solvents such as confidential list have potential for being released into this media.

6.9.2.2(a) Control Technology and Efficiency

Two step biological wastewater treatment with a biodegradation capacity of 30-95% of compounds with respect to dissolved oxygen content (DOC) for the combined effluents of Ciba-Geigy and Roche; regulations call for a

85% elimination. The limit for total suspended substances of 20 mg/l is also met. Essentially the two steps of wastewater treatment as performed in Ciba-Geigy/Roche plant consist of a physical step including sedimentation and floatation as well as a biological treatment in an aeration tank.

The wastewater of each step of the process is controlled with respect to the relevant regulations which call for compliance with limits for 52 individual parameters. In order for permission for a discharge of the sewage to the official industrial wastewater treatment plant to be granted, limits for all parameters must be achieved. Furthermore, samples of the wastewater are routinely examined in an in-house pilot plant for wastewater treatment prior to discharging to the industrial wastewater treatment plant. The biodegradability of the wastewater of the total manufacturing process is at least 90% with respect to the DOC. The outlet of the wastewater treatment plant is in compliance with all relevant regulations; it is regularly and systematically controlled by local authorities. The treated clarified effluent is discharged into the Rhine River.

The regulation in force is the ordinance for wastewater discharge which is based on the Swiss Federal Law relating to protection of water against pollution. The ordinance considers a total of 52 parameters (e.g. temperature, color, odor, total suspended substances, pH-value, O_2-content, inorganic substances individual heavy metals, phosphorous-content, DOC, etc.) for which the corresponding indicated limits must be observed. The 52 parameters apply on the one hand to the discharge of the sewage to the wastewater treatment plant, on the other hand to the treated clarified effluent. The ordinance calls for a 85% biodegradation of the wastewater and sets a limit for total suspended substances of 20 mg/l. For some of the 52

parameters the local authorities can set alternative limits based on local situations, thus, for suspended substances a limit of 40 mg/l is applicable in the region of Basel-Stadt.

The discharge of the sewage to the wastewater treatment plant itself is not subject to an official permit but is within the scope of the responsibility of the company. The outlet of the wastewater treatment plant is controlled daily by the staff of the plant. The controls comprise the 52 parameters prescribed by the regulations as described above. The results of the investigations are submitted regularly to the governmental department for water protection in the course of the quarterly reports. In addition, a mixed sample of the wastewater is saved every day for official inspection if required. Furthermore, as mentioned previously, the outlet of the wastewater treatment plant is regularly controlled by the authorities with respect to all parameters of the corresponding regulations.

F. Hoffmann-La Roche Ltd, Basel, Switzerland will incinerate solid/liquid waste of Saquinavir mesylate drug substance and drug product including rejected batches and expired drug product. The permit number for the incinerator at the F. Hoffmann-La Roche Ltd, Basel Switzerland is 27010057 which was issued on December 22, 1993 with an expiration date of December 31, 1995. The permit is issued by the department for water protection of the canton Basel-Stadt. A copy of the actual permit is included in Appendix H.

6.9.2.3 Solid/Liquid Waste

Solid/Liquid wastes from the Saquinavir mesylate manufacturing process consist primarily of solvents which are partially recovered, some organic byproducts.

6.9.2.3(a) Control Technology and Efficiency

The bulk of the solvents (confidential) is recovered in distillation units of Roche Basle. Residues, inseparable solvents and solid wastes are incinerated in our own incinerator which is equipped with flue gas scrubbing. The flue gas emitted is in accordance with the relevant regulations. Confidential chemical is recycled in France to obtain a confidential chemical which is again used in the synthesis of Saquinavir mesylate.

Roche Basle holds a permit for the acceptance and treatment of special wastes according to the Swiss Ordinance on movements of special wastes. This permit is concomitant with a regulatory inspection by Swiss authorities with respect to:

- the total emissions involved in the special waste treatment;
- the technical equipment involved;
- auxiliary equipment;
- the professional competence of the laborers.

Eighty percent of the special wastes of the Roche Basle facility are treated in the company's own plant. The rest is sent offsite for thermal destruction to a specialized company which is also in the possession of a permit for acceptance and treatment of special wastes and is subject to the same official inspections.

All activities relating to the treatment of special wastes are in accordance with the corresponding regulations.

The following two regulations are related to the disposal of wastes:

Ordinance on movements of special wastes

Technical ordinance on wastes

The ordinance on movements of special wastes regulates the disposal, transport and acceptance of special wastes including import, export and transit. The acceptance and handling of special wastes are subject to a permit from the governing authority. The ordinance contains a list of special wastes divided into 14 categories according to which the contractor must classify and declare his special wastes for further treatment.

The technical ordinance on wastes contains regulations for the diminution, the treatment and the utilization of wastes. In particular, the mode of treatment of the individual type of wastes is prescribed and the requirements for the working of incineration equipment and landfills are specified.

6.10 Milling of Ro 31-8959/008 England

6.10.1 Industrial Hygiene Controls

The milling process for Saquinavir mesylate is fully compliant with United Kingdom COSHH regulations. All process operatives are protected in accordance with internal procedures and receive periodical medical examination.

6.10.2 Substances Expected to be Emitted- Milling of Ro 31 -8959

During the milling process some material may be released in the aqueous, terrestrial and air phase. The section below describes potential loss of materials(s), containment devices utilized and their efficiencies, applicable permit information and other relevant regulatory information.

6.10.2.1 Air Emissions

The only potential air emission is fugitive dust. All processes are carried out in controlled areas with an air handling system providing a minimum of 25 air changes per hour.

All process air and processing suite air handling system discharge is first filtered through pre-filters (specification Eurovent 4/5) then nepa-filters (99.99% arrest at 0.3 microns) before discharge to atmosphere.

The Environmental Protection Act (1990), with air pollution specifically controlled by the Environmental Protection (Prescribed Processes ans Substances) Regulations 1991.

6.10.2.2 Wastewater Emissions

Wastewater discharges are generated by equipment and area washings after completion of the process.

the normal procedure to mitigate release of saquinavir mesylate powder into wastewater is to dry run the mill (without powder) for 10 minutes, followed sweeping the mill and all other equipments. All residues placed into a polythene bag for shippment to F. Hoffmann-La Roche Ltd., Basel, Switzerland.

Suspended solids are removed via a settlement system and effluent is discharged to the foul sewer in line with

Local Water Authority consent. The Local Water Authority regularly samples and monitors discharges to ensure compliance with consent.

Controlled by local Water Authority consent, with limits set by individual consent.

6.10.2.3 Solid/Liquid Wastes

All solid/liquid waste (if applicable) will be returned to Roche Basel.

6.11 Milling of Ro 31-8959/008:

Milling Ro 31-8959/008 will be carried out at A compliance statement signed by foreign regulatory authorities is included in Appendix G.

6.12 Manufacture of Drug Product: Hoffmann-La Roche Inc., Nutley,NJ

The Invirase drug product consists of capsules containing a combination of the active drug substance Saquinavir mesylate and excipients produced at the Hoffmann-La Roche Inc. plant in Nutley, New Jersey. All manufacturing operations are carried out under carefully controlled conditions and in compliance with applicable environmental regulations of the U.S. Environmental Protection Agency (USEPA) and New Jersey Department of Environmental Protection and Energy (NJDEPE). Emission of pollutants into the air and water and disposal of solid waste for the Hoffmann-La Roche Nutley facility are regulated to a high degree by the State of New Jersey, specifically in Title 7, Environmental Protection, of the New Jersey Administrative Code. A statement of compliance is included in Appendix E.

6.12.1 OSHA Regulated Compounds

The chemical substances used as ingredients in the manufacture of invirase capsules are listed in confidential Appendix C and D. Copies of available MSDS's are included in confidential Appendix C and D

6.12.2 Substances Expected To Be Emitted

During the manufacturing process some material may be released in the aqueous, terrestrial and air phase. The section below describes potential loss of materials(s), containment devices utilized and their efficiencies, applicable permit information and other relevant regulatory information.

6.12.2.1 Air Emissions

Air emissions consist of minor amounts of pharmaceutical dust (active ingredients plus excipients) lost during loading of dry ingredients into the blending and capsule filling equipment. Emission of particulate matter is controlled by means of fabric filter dust collectors.

Air emissions in New Jersey are regulated under N.J.A.C. 7:26-1 et seq., the Bureau of Air Pollution Control portion of the New Jersey Administrative Code. These regulations include subchapters governing allowable emissions of particulate matter and volatile organic substances from manufacturing processes, as well as setting forth the requirements for obtaining permits to construct or alter process equipment.

The rooms where the various types of equipment are used in the blending, milling and capsule filling operations for the production of Invirase drug product serviced by dust collectors, of the fabric filter type. The general purpose room ventilation systems predate the current air permit system and are thus "grandfathered." All equipment operates in compliance with current requirements for particulate emissions.

The level of dust in the processing areas during product blending and capsule

manufacturing operations will be controlled by local exhaust ventilation and general room ventilation. In addition, employee exposure levels will be minimized by the use of personal protective equipment such as respiratory protectors, if required.

6.12.2.2 Wastewater Effluent:

The wastewater from Invirase capsule manufacturing process consists mainly of equipment washdowns. The wastewater from the blending and capsule filling operations contains residual amounts of active ingredients along with excipients and other components used in the manufacture of the drug product.

Wastewaters from the Invirase drug product manufacturing process are combined with wastewater from other manufacturing processes and discharged through a pretreatment system to the Passaic Valley Sewerage Commission (PVSC) treatment plant (a POTW) under PVSC Permit Number 24402882 (expiration date April 14, 1996). The State of New Jersey regulates the Roche/Nutley pretreatment facility as a significant industrial user and has issued non-contact cooling water and storm water discharge permit number NJ 0034185 (expiration date January 31, 2000) under the New Jersey Pollutant Discharge Elimination System (NJPDES) regulations.

Wastewater discharges from the Invirase manufacturing process will be in compliance with the above referenced regulations and the conditions of both operating permits.

6.12.2.3 Solid Liquid Wastes:

Based on the experience with similar products for operations involved with the production of capsule products, approximately 95 percent of the materials go to form finished product. Of the 5 percent loss, the majority is in the form of solid waste, either material cleaned up from the blending, granulating and capsule filling operations or the fabric dust collectors which service them. The releases to the environment consist of the small quantity of material which is washed from the various types of equipment after the majority of the material has been removed by normal means such as dry vacuuming.

Solids for disposal consist mainly of broken and rejected capsules. Solid wastes containing Invirase that are not sewered (see above) will be collected and disposed in a lined industrial landfill or incinerated.

Hoffmann-La Roche Inc., Nutley, New Jersey will incinerate any production rejected batches of Saquinavir mesylate drug substance and drug product in a medical waste incinerator located on site. The medical waste incinerator air permit number is 113190 with expiration date of June 17, 1995 which has been granted conditional 90 day extension.

All solid/liquid waste of Saquinavir mesylate will be either incinerated on the Nutley site and/or sent to an outside contractor for industrial landfill. At present, two out side contractors are being retained for industrial landfill. The mailing addresses and permit information for these two contractors are as follows

- Grand Central Sanitary Landfill Inc.
  1953 Pen Argyl Road
  R D # 1 Box 211
  Pen Argyl Pennsylvania 18072

  PERMIT NUMBER 100265
  ISSUED ON 11/13/80

- CWM Chemical Services, Inc.,
  1550 Balmer Road
  Model City, New York 14107

  PERMIT NUMBER NY 0072061
  ISSUED ON 10/8/93 with an expiration date of 10/1/98

  A copy of the permit for medical waste incinerator located at Hoffmann-La Roche Inc. Nutley, New Jersey and a copy of the permit for Grand Central Sanitary Landfill, Inc and CWM Chemical Services Inc., are included in Appendix H

  Disposal of solid waste and hazardous waste is controlled under the NJDEPE regulations, N.J.A.C. 7:25-1 et seq. Disposal and processing of solid/liquid waste from the Invirase process will be in compliance with the NJDEPE waste regulations referenced above.

7. FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT:

Summary of Absorption and Metabolism of Saquinavir mesylate

The absorption of Saquinavir mesylate after oral administration is less than 20% in the rat and a maximum estimate of 30% was found for man. The oral absorption was dose dependent. The mean recovery of radioactivity in 72 hours after oral administration to rats was 88 ± 23%, distributed between urine (5.1 ± 1.3%), bile (11.3 ± 5.3%) and feces (70.5 ± 21.4%) indicating rather rapid clearance from the body Saquinavir mesylate is characterized by low absorption and bioavailability. In human volunteers, approximately 89% of dose excreted in urine (1%) and feces (88%) following oral administration at 600 mg/kg dose level. Saquinavir mesylate is characterised by low absorption and bioavailability.

The bioavailability of Saquinavir mesylate following oral administration range from approximately 4.1,8, ar ercent in rats, rabbits, dog and marmoset, respectively.

Billiary excretion of drug-related accounts for greater than 95% of the dose after intravenous administration to rats, rabbits and man.

The metabolic profile of Saquinavir mesylate has been investigated in the bile, plasma and microsomes from rats and in microsomes from other species, including man. There is good correlation between the billiary and microsomal metabolites and the profiles in microsomes are qualitatively similar across all species. After oral administration in rats, there were seven major drug-related zones detected by HPLC which is equivalent to about 90% of the recovered activity in the bile. One of the metabolite is presumed to be the product of oxidation of one of the methyl group of the tertiary butyl group to a carboxylic acid. All of the other metabolites arose by hydroxylation of the decahydroisoquinoline ring.

In a separate study, bile was collected from male rats which had been dosed intraperitoneally at 200 mg/kg. A plethora of metabolites was produced; HPLC analysis indicate that there were at least 50 metabolites and that none amounted to more than 5% of the recovered drug-related material.

The in vitro microsomal metabolic profile of Saquinavir mesylate in man is qualitatively similar to that of other animal species studied. Approximately, seven metabolites and the parent drug was detected in human microsomes study.

Metabolic profile in the urine and feces has not been determined. However, based on the poor absorption (30%) in man and in vitro microsomal study suggest that no one metabolite is likely to exceed 10% of the total excreted material.

The above summary of absorption and metabolism have been prepared from a technical report entitled "Saquinavir mesylate of Absorption and Disposition in Animals" A copy of the entire report is included in Appendix I.

The present NDA for Invirase is for the therapeutic use of it in the treatment of AIDS and AIDS related complex. This drug will be made available for therapeutic use in oral formulations through physician prescriptions

7.(a) Air:

The active drug substance is manufactured by F. Hoffmann-La Roche Ltd., Basle, Switzerland in accordance with applicable laws. The final dosage form will be manufactured at our Basle facility and/or at Hoffmann-La Roche Inc. facility located in Nutley, New Jersey. Potential air emissions consist of minor amounts of pharmaceutical dust (active ingredient plus excipients) lost during loading of dry ingredients into capsule filling equipment. Emission of particulate matter is controlled by means of fabric filter dust collectors. All equipment operates in compliance with current requirements for particulate emissions. Based on the experience with similar products for operations involved with the production of capsules, approximately 95 percent of the materials go to form finished product. Based on the estimated maximum annual production volume provided to the FDA in the confidential environmental assessment, only a small amount is expected to be released in the air. Of the 5 percent expected release, the majority is in the form of solid waste due to material cleaned up from equipment or the fabric filter dust collectors which service them. Control efficiencies for fabric filter dust collection is in excess of 99.9%, which will further reduce the amount expected to be released in the air. The waste will be either incinerated or disposed in a lined industrial landfill. Therefore, it is not expected to be released in the air in significant amounts to cause detrimental effects during the manufacturing of Invirase drug product. The workers will be protected by engineering control and/or use of personal protective equipments.

Invirase will be made available through physician prescription only. The drug is not likely to b released in the air through usage except accidental spill (breakage of capsules) situations. Even in such a remote situation the drug is not likely to be released into the ambient air due to it's physical form and high melting point. A minute environmental release of the drug is also expected to be dispersed in the ambient air so that concentrations per cubic meter of air would be extremely small (probably in ppb range) to cause any adverse environmental impact.

There are no direct acute and subchronic toxicological studies available to evaluate the toxicological effects of low level inhalation exposure to Saquinavir mesylate However, in a number of clinical studies via oral doses of this drug have been well tolerated by human volunteers. Animal studies also indicate relatively low toxicity. For example, acute oral LD50 to rat is greater than 5000 mg/kg. It is not a teratogenic, mutagenic or reproductive toxicant. (MSDS, Appendix B).

Based on available pharmacological and toxicological studies as well as physical chemical properties and production volume, the drug substance

is not likely to persist in the ambient air to cause any significant adverse environmental impact.

7.(b) Impact on Fresh Water, Estuarine and Marine Eco Systems:

The wastewater from Saquinavir mesylate capsules manufacturing process consists mainly of equipment washdowns. The wastewater from the capsule filling operations contains residual amounts of active ingredients along with excipients used in the manufacturing of drug products. As mentioned above under air, (item 7, a) most of the loss will be in the form of solid waste. The releases to the water consist of the small quantity of material which is washed from the various types of equipment after the majority of the material has been removed by normal means such as, dry vacuuming.

Based on the estimated maximum annual production volume provided to the FDA in the confidential environmental assessment, only small amounts (probably less than 0.001 %) is expected to be released in the water. The wastewater effluent from the Invirase capsule filling process is combined with wastewater from other manufacturing processes and discharged through a pretreatment system to the Passaic Valley Sewage Commission (PVSC) treatment plan (a POTW), which is equipped to process 300 million gallons per day. The expected released drug from the manufacturing site would be diluted to a concentration below that of significant concern.

Following therapeutic use, minute concentrations of the drug and it metabolites will be released into sewage through fecal and urinary excretions.

In order to evaluate the impact of the very low concentration of Saquinavir mesylate expected to enter the aquatic environment, we determined the concentrations of Saquinavir mesylate that must be attained before toxic effects are observed. In aquatic species the following studies were conducted to evaluate its effects.

1. Daphnia magna acute toxicity:

The acute effects of Saquinavir mesylate on the fresh water invertebrate water flea, Daphnia magna were evaluated under static conditions according to the FDA Environmental Assessment Technical Assistance 4.08. Based on the results of the range finding study, nominal

concentrations selected for definitive tests were 13.0, 21.6, 36.0, 60.0, and 100 mg/L. Twenty neonates were exposed to each concentration for 48 hours. The 48 hour median effective concentration (EC50), the concentration of Saquinavir mesylate estimated to result in immobilization or death to 50 percent of the test population was calculated to be greater than 100 mg/L. The No Observed Effect Concentration (NOEC) was 36 mg/L. This study was performed according to the FDA Good Laboratory Practices (GLP) Guidelines. The detailed report of this study is provided in Appendix J.

2. Freshwater Fish Acute Toxicity (Rainbow Trout):

The acute toxicity of Saquinavir mesylate to Rainbow Trout (Oncorhynchus mykiss), a cold-freshwater fish was conducted under static condition according to the FDA Environmental Assessment Technical Assistance 4.11. Twenty fish per each treatment concentration having a mean weight of 0.37± 0.11 gram were exposed for 96 hours. Actual measured concentrations by High Pressure Liquid Chromatography (HPLC) ranged from 0 to 38 mg/L. Due to limited water solubility of Saquinavir mesylate precluded from testing at a higher concentrations. The 96-hour median lethal concentration (LC50), the concentration of Saquinavir mesylate estimated to be lethal to 50 percent of the test population was calculated to be greater than 38 mg/L. The NOEC was 38 mg/L. This study was conducted according to the FDA GLP Guideline. The detailed report of this study is included in Appendix K.

3. Microbial Growth Inhibition Study:

The Minimum Inhibitory Concentrations (MIC) of Saquinavir mesylate were determined according to the FDA Environmental Assessment Technical Assistance 4.02. The organisms utilized in this study were free living nitrogen fixing bacteria (Azotobacter vinelandii), soil bacteria (Pseudomonas putida), blue-green alga (Anabaena flos-aquae), ascomycete (Fusarium acuminatum), and mold (Aspergillus niger). The MIC was 312 mg/L for the blue-green alga. No growth inhibition was observed for any of the other test species at or below 312 mg/L. This study was conducted according to the FDA GLP Guideline. The detailed report of this study is included in Appendix L.

4. Activated Sludge Respiration Inhibition Study:

Saquinavir mesylate was tested in the activated sludge respiration inhibition test according to the Organisation for Economic Co-operation

and Development (OECD) Guideline reference #209. The respiration rate of an activated sludge and synthetic sewage aerated for 3 hours in the presence of test substance was compared to the respiration rate of control to which no substance was added. The respiration rate was measured in the presence of Saquinavir mesylate at concentrations ranged from 0 to 40 mg carbon/L. The highest level of inhibition observed was 29.4% at 40 mg carbon/L (58.85 mg/L total weight). The EC50 value is estimated to be greater than 40 mg carbon/L. This study was conducted according to the FDA GLP Guideline. The detailed report of this study is included in Appendix M.

5. Aerobic Biodegradation in Water:

Saquinavir mesylate was tested for biodegradability in an aqueous medium at a test concentration of 10.0 mg carbon/L according to the FDA Environmental Assessment Technical Assistance 3.11. The aqueous medium consisted of a composite inoculum of soil filtrate and secondary effluent, mineral salt media and yeast extract. The production of carbon dioxide was measured at various intervals over a period of 28 days. Less than 1% of the initial dose of Saquinavir mesylate was mineralized to carbon dioxide in 28 days. This study was conducted according to the FDA GLP Guideline. The detailed report of this study is included in Appendix N.

6. Freshwater Green Alga Acute Toxicity Study:

The freshwater green alga (Selenastrum capricornutum) acute toxicity study was conducted under static conditions according to the FDA Environmental Assessment Technical Assistance 4.01. The measured concentrations by HPLC ranged from 0 to 20.5 mg/L. Dimethylformamide was used as a solvent. Solvent controls as well as non-solvent controls were incorporated in the study. The NOEC and MIC, based upon the largest specific growth rate were 10.4 and 20.5 mg/L respectively. This study was conducted according to the FDA GLP Guideline. The detailed report of the study is included in Appendix O.

7. Solubility

The solubilities were measured in various solvents. An excess of the compound was roller mixed with 5 ml of solvent for 24 hours at room temperature (21° C). The solution was then centrifuged and the organic solvents filtered through a 0.45 micrometer filter disc. The aqueous solvents were not filtered in order to avoid losses due to adsorption of

the compound, however the solutions appeared to be clear. An appropriate dilution in methanol was made to all the resultant solutions. A measured volume from these two solutions was evaporated then redissolved in methanol to avoid interference with UV absorbance. The solubilities were determined by comparing the absorption of the diluted test mixture with that of a standard solution.

8. Dissociation Constant

The dissociation constant (pKa_2) of the decahydroisoquinoline-3-carboxylic acid t-butylamide was measured by UV spectrophotometry in 10 % ethanol/water. The dissociation constant (pKa_1) of the quinoline was determined by measuring the change in UV absorption of a fixed weight of sample in various buffer solutions of known pH or Ho values.

9. Partition Coefficient

The drug substance was dissolved in n-octanol previously saturated with water. An aliquot (10 ml) of this solution and 20 ml of the aqueous phase previously saturated with water was pipetted into a 50 ml centrifuge tube and shaken on a horizontal shaker for 24 hours at ambient temperature. The centrifuge tube containing both layer was centrifuged to separate the layers. The concentration of Ro 31-8959 in each layer was then determined by High Pressure Liquid Chromatography (HPLC).

The Maximum Expected Emitted Concentration (MEEC) of Saquinavir mesylate and/or its metabolites in US domestic waste may be estimated from the assumed daily average per capita amount of Saquinavir mesylate used and the estimated average per capita volume of water containing these materials. The estimated MEEC value is confidential concentrations in ppm. The estimated Expected Environmental Concentration (EEC) is confidential concentrations in ppm with the assumption of depletion due to aquatic biodegradation processes assumed to be zero as a worst case scenario. The estimation of MEEC and EEC is shown in Appendix P. Based on the aquatic effects studies, the EEC value is several fold lower than MIC value of 20.5 mg/L for green algae the most sensitive species. Therefore, it is concluded that this drug is not likely to cause any deleterious adverse effects to aquatic organisms through its usage.

The maximum Biological Oxygen Demand (BOD) corresponding to the estimated MEEC may be estimated from the amount of oxygen required for conversion of C to CO2 and H to H2O. One mole of Saquinavir mesylate ($C_{38}H_{50}N_6O_5$) requires an additional 48 moles O2 to produce

38 moles CO2 and 25 moles H2O. The mg/L BOD may be obtained by from the following:

$$BOD = (MEEC)\ (48)\ (MWO2)\ /(MW\ C_{38}\ H_{50}\ N_6\ O_5)$$

$$BOD = (confidential\ concentrations\ mg/L)\ (48)\ (32)/(670)$$

$$BOD = confidential\ mg/L$$

The BOD arising from the use of Saquinavir mesylate is insignificant compared to the normal BOD, 300-500 mg/L, of domestic sanitary waste.

7.(c) Terrestrial Ecosystems:

The drug is not likely to be released in terrestrial ecosystems either through usage or during the manufacturing process. All solid/liquid waste generated during manufacturing will be incinerated and/or disposed in a lined industrial landfill. The drug and its metabolites will primarily be released in the aqueous environment through usage. Therefore it is not likely to cause any detrimental effects in terrestrial ecosystems.

Animal studies have indicated relatively low toxicity. The acute oral LD50 for rats is greater than 5000 mg/kg indicating relatively low mammalian toxicity. Saquinavir mesylate is neither a teratogen, mutagen nor a reproductive toxicant (Appendix B, MSDS). In clinical studies via oral doses, this drug was well tolerated by human volunteers. The elimination of the drug via feces and urine was rapid. Therefore, it is expected that the drug is not likely to cause significant impact in the terrestrial ecosystems if released accidentally and/or due to minute (PPT) range) concentration in the reclaimed sludge.

Soil adsorption/desorption studies were conducted on Saquinavir mesylate according to the FDA Environmental Assessment Technical Assistance 3.08. Three different types of soil (loam, silt loam and clay loam) were used in this study. The calculated kd values were 371.0, 127.2, and 133.0 for silt loam, clay loam and loam, respectively. The calculated K_{oc} values were 10,692, 22,919 and 13,711 for silt loam, clay loam and loam, respectively. Therefore, under the conditions and criteria of the test, Saquinavir mesylate was characterized as being immobile in all soils tested. This study was concluded according to the FDA GLP Guidelines. The detailed report of this study is included in Appendix Q.

The earthworm subacute toxicity study was conducted according to the FDA Technical Assistance Document guideline 4.12. The test organisms utilized in this study were nightcrawlers (Lumbricus terrestris).

A preliminary range finding study was conducted with saquinavir mesylate at doses up to 1000 mg/kg. No significant mortality occurred at any dose levels tested.

Because of lack of significant mortality in a preliminary range finding study at doses up to 1000 mg/kg, a definitive study was conducted at a single dose level of 1000 mg/kg. At this dose level there was no mortality observed during the test duration. Therefore, it is concluded that the 28-day LC_{50} of saquinavir mesylate to earthworm is greater than 1,000 mg/kg.

After patient use, saquinavir mesylate will be discharged into sewage and wastewater treatment plants. Given the high $logK_{ow}$ values at pH 6.9 and 9.4 and the high K_{oc} values for various soil types, saquinavir mesylate may sorb to sludge in wastewater treatment plants. As the most common sludge disposal method in the United States is incineration, any saquinavir mesylate bound to sludge would also be incinerated.

Upon discharge from wastewater treatment plants into the aquatic environment, saquinavir mesylate may be expected to sorb to sediment and soil particles and thus partition into the terrestrial environment. Although it does not biodegrade rapidly, saquinavir mesylate may be expected to bind tightly to sediment and soil and thus its bioavailability would be lowered. Furthermore, saquinavir mesylate is of low toxicity to earthworms and to representative soil microorganisms. The 28 day LC_{50} of saquinavir mesylate to earthworm (nightcrawler) was greater than 1000 mg/kg. No mortality occurred at doses upto 1000 mg/kg during the study period. The minimum inhibitory concentrations (MIC) of saquinavir mesylate to various soil microorganisms was greater than 312 mg/L. Saquinavir mesylate which partitions into the terrestrial environment after patient use is not expected to have an adverse impact on terrestrial organisms.

Based on the above discussion and study results, it is concluded that the drug is not likely to cause any significant detrimental effects in terrestrial ecosystems.

GENERAL DISCUSSION

In a aerobic biodegradation in water study, saquinavir mesylate was not readily biodegradable indicating low potential for removal or depletion from the environment. Based on its structure, it is expected that the hydrolysis or photolysis of saquinavir mesylate

will play a insignificant role in ultimate removal or depletion from the environment.

Saquinavir mesylate is not likely to be released in air after patient use. During manufacturing it is not likely to be released in the air in significant amounts because of various control measures are in place. Therefore, it is not likely to be in air in significant amounts to cause any harm to the environment.

The wastewater from saquinavir mesylate capsules manufacturing process consists mainly of equipment washdowns. The wastewater may contain residual amounts of saquinavir mesylate along with other excipients. After patient use, saquinavir mesylate and its metabolites will be discharged into sewage and wastewater treatment plants.

Saquinavir mesylate is of low toxicity to various aquatic organisms. The lowest no observed effects level (NOEL) is 10.4 mg/l for green algae. The NOEL value is greater than the water solubility of saquinavir mesylate, which indicate no significant toxicity to aquatic organisms

The maximum expected Environmental Concentrations (MEEC) is 7.6×10^{-4} ppm. The results of effects studies indicate that the MEEC of saquinavir mesylate arising from product use should not have an unfavorable environmental impact on aquatic life, since the lowest aquatic NOEC is greater than the MEEC by a factor of approximately 13,000

Saquinavir mesylate may be sorb to sludge in wastewater treatment plants. As the most common sludge disposal method in the United States is incineration, any saquinavir mesylate bound to sludge would also be incinerated.

No direct release of saquinavir mesylate into the terrestrial environment is expected through manufacturing and after patient use. Upon discharge from wastewater treatment plants, saquinavir mesylate may be expected to sorb to sediment and soil particles and thus partition into the terrestrial environment. The results of soil adsorption/desorption study indicate that the saquinavir mesylate was tightly bound to various soil types. Therefore, it is not expected to be bioavailable Furthermore, saquinavir mesylate is of low toxicity to earthworm (LC_{50} greater that 1000 mg/kg) and to representative soil microorganisms (MIC greater than 312 mg/L).

Based on its lipophilicity and slow biodegradation of saquinavir mesylate, it is possible that it may bioaccumulate. However, soil adsorption/desorption study indicate that the saquinavir mesylate was tightly bound to various types soils. Therefore, may sorb and bind tightly to sediment soil particles and thus not be bioavailable to cause any detrimental effects in the environment. Furthermore, because of its large molecular weight, it is not expected to cross biological membranes in significant amounts. Saquinavir mesylate is rapidly metabolized in the body.

Saquinavir mesylate is of low toxicity to aquatic organisms, soil organisms and terrestrial organisms. For example, the NOEC for green alga is 10.4 mg/L which is around maximum or higher than the aqueous solubility of saquinavir mesylate. The MIC value of saquinavir mesylate to various micro-organisms is greater than 312 mg/L. The 28 day LC_{50} of saquinavir mesylate to earthworm is greater 1,000 mg/kg. The Maximum Expected Environmental Concentrations (MEEC) value is 7.6×10^{-4} which is very low, thus providing higher safety factor of approximately 13,000 between toxicity (NOEC) and MEEC) value. Therefore, bioaccumulation of saquinavir mesylate is expected to be too low to cause any detrimental effects in the environment.

Based on the above discussion, it is concluded that the saquinavir mesylate levels are not expected to cause any harm to the environment and to the organism co-habitat in the ecosystem.

8. ENVIRONMENTAL EFFECTS OF RELEASED SUBSTRATES:

The primary impact from the use of Saquinavir mesylate will be the disposition of the drug subsequent to oral administration. The discharge of human excreta containing drug product residues and/or its metabolites will be handled by conventional sewage disposal systems in accord with federal, state, and local regulations. Furthermore, Saquinavir mesylate will be prescribed in milligram quantities by a licensed practitioner, its distribution will be consequently limited. Therefore, its use will not be indiscriminate and will not adversely affect, to a significant extent, the quality of the environment. A secondary environmental consequence results from the minor discharge of manufacturing pollutants to air and water during manufacturing. Such discharge will also be in accord with all federal, state, and local requirements.

8.1 Air:

The physical-chemical and environment fate and effects data reported in item 7, as well as the controls exercised at the site of manufacture of drug substance and drug product, indicate that insignificant concentrations of Saquinavir mesylate in ambient air will result from the use and/or disposal of Saquinavir mesylate. The potential health effects on humans from exposure to Saquinavir mesylate are limited to occupational exposure. As mentioned under OSHA regulated compounds, appropriate engineering and protective equipment controls will be used to protect employees

8.2 Freshwater Estuarine and Marine Ecosystems:

In order to evaluate the impact of the very low concentration of Saquinavir mesylate expected to enter aquatic environment, number of studies described in item 7, were conducted. The following is the summary of environmental effects data.

Daphnia Magna (static)	36	> 100
Rainbow Trout (static)	38	> 38
Microbial Inhibition. (4.02)		
Algae		312
Other species		> 312
Activated Sludge		> 58.8
Green Algae	10.4	20.5

The estimated MEEC value is confidential concentrations ppm or confidential mg/L The estimated EEC value is confidential concentrations mg/L with assumption of depletion by aquatic system is zero The results of effects studies reported above indicate that the EEC of Saquinavir mesylate arising from product use should not have an unfavorable environmental impact on aquatic life, since the lowest aquatic NOEC is greater than the EEC by a factor of approximately 13,000.

8.3 Terrestrial Ecosystem

As mentioned under item 7, the exposure to human or animals is not likely to occur during the manufacturing or through usage of the drug. Furthermore, the preliminary results of the earthworm subacute toxicity study indicate that the LC50 is greater than 1000 mg/kg. The soil adsorption/desorption study suggest that it is immobile. Based on these studies it is expected that no significant detrimental effects are likely to occur on terrestrial life.

9. USE OF RESOURCES AND ENERGY:

The maximum annual production of the drug substance for the U.S. market over the five year period following introduction is provided to the FDA in the confidential volume.

The annual production volume for the Chemical Operations Department and the total utilities and energy cost for the year 1994 for the Hoffmann-La Roche Inc. Nutley facility are provided to the FDA in the confidential volume. Based on these figures and the estimated maximum 5-year production volume of Saquinavir mesylate drug product, the additional utilization due to the manufacture of the Saquinavir mesylate drug product will be 0.001% of the total.

The facility used for production of Saquinavir mesylate is already committed to the production of other Pharmaceuticals and Vitamins and Fine Chemicals. There are no effects expected upon endangered or threatened species due to the proposed action. There are no effects expected upon property listed in or eligible for listing in the National Register of Historic Places.

10. MITIGATION MEASURES

Environmental impacts associated with the production of Saquinavir mesylate will be avoided or mitigated by the use of appropriate control measures in accord with all federal, state, and local regulations. Air emissions control devices includes vent condensers, scrubbers and fabric filter dust collectors. Environmental impacts associated with the disposition of drug substance and/or metabolites following consumption in humans will be mitigated by conventional wastewater treatment plants. All rejected Saquinavir mesylate drug product will be disposed in a lined industrial landfill or incinerated. Unused or out-of-date product is returned to Roche/Nutley for credit and disposed in the same manner.

Waste minimization is considered in the design of Hoffmann-La Roche processes to the extent possible while maintaining the quality and purity of the manufactured drug substance. Solvents are recovered and reused within the same process or for a different product depending on quality control specifications. Yield maximization is an important factor at all stages of pilot plant and process development.

Chapter 7:1E of the New Jersey Administrative Code covers Discharge of Petroleum and other Hazardous Substances. The Hoffmann-La Roche Nutley facility is classed as a major facility under this regulation and as such is required to maintain plans for Discharge Prevention, Containment and Countermeasures (DPCC) and Discharge, Cleanup and Removal (DCR) acceptable to the New Jersey Department of Environmental Protection and Energy (NJDEPE). In addition to the physical facilities for containment of spills which are described in the DPCC plan, three emergency squads have been established at the Nutley site:

1. Roche Environmental Response Squad (ERS)

2. Roche Fire Brigade

3. Roche Medical/Heavy Rescue Squad

The squads consist of a total of over 90 volunteers from various departments within Roche who respond to emergencies and actively participate in monthly training drills, as well as semiannual joint emergency drills with Clifton and Nutley Local Emergency Planning Committee's (LEPC).

The Roche Environmental Response Squad (ERS) currently consists of 28 highly trained individuals who deal immediately and effectively with air, land, and water spills of hazardous substances, at the Nutley plant. The team also lends its assistance to our LEPC's and other Roche facilities. It consists of representatives from plant-wide activities maintaining a balance of technical and skilled personnel from various plant activities, i.e., tank farm, boiler operations, wastewater treatment, chemical processing, warehousing, and laboratories.

The ERS works closely with the other emergency squads and the Chemical Production Department (CPD) personnel in order to effectively deal with environmental emergency situations.

11. ALTERNATIVES TO THE PROPOSED ACTION:

Alternatives available to the FDA include non-approval and notification of intent to prepare an Environmental Impact Statement (EIS) in addition to the approval of the proposed action through the issue of Finding of No Significant Impact (FONSI). We believe that the latter action, issuance of a FONSI, is fully justified by this Environmental Assessment. Manufacturing operations will be in compliance with the regulations of the applicable governmental agencies. Releases of Saquinavir mesylate to the environment will be mitigated as discussed in Item 10. Fate and effects testing - described in Sections 7 and 8 support the position that the manufacture and use of Saquinavir mesylate will not produce an adverse effect on the environment.

Approval of the proposed action will make available to the physician a significantly valuable, potentially life-saving drug, and improving the quality of life of AIDS patients. The therapeutic benefits of which are discussed elsewhere in this NDA.

12. LIST OF PREPARERS:

P.V. Shah, Ph.D
Senior Industrial Toxicologist
Corporate Environmental and Safety Affairs.

(See Curriculum Vitae - Appendix R)

13. CERTIFICATION:

The undersigned official certifies that the information presented is true, accurate, and complete to the best of the knowledge of the persons responsible for preparation of the environmental assessment.

Jack S. Kace 9/6/95

Jack S. Kace, Eng Sc D
Vice President and Director
Corporate Environmental and Safety Affairs

14. REFERENCES:

All references cited are to Hoffmann-La Roche internal reports which are included as appendices in the confidential version of the Environmental Assessment

15. APPENDICES:

A. The Synthetic Flow Chart - Ro 31-8959/008.

B. Physical - Chemical Property Data and Material Safety Data Sheet (MSDS) for Saquinavir mesylate

C. List of Chemical Substances along with CAS No. associated with manufacture of Drug Substance and copies avalable MSDSs (confidential)

D. List of Chemical Substances along with CAS No associated with manufacture of Drug Product and copies of available MSDSs (confidential).

E. The maximum Projected Annual Production and Energy Utilization Data.

F. Compliance Statements signed by a High Ranking Official from various Companies.

G. Compliance Statements from Foreign Regulatory Authorities.

H. Copy of Incinerator and Industrial Land fill Permits for F. Hoffmann-La Roche Ltd., Hoffmann-La Roche Inc., and Contractors

I. Saquinavir (Ro 31-8959): Technical Summary of Absorption and Disposition in Animals.

J. Test Report Ro 31-8959: Acute Toxicity to the Water Fleas Daphnia magna under static conditions.

K. Test Report Ro 31-8959 Acute Toxicity to Rainbow Trout, Oncorhynchus mykiss, under Static Conditions.

L. Test Report Ro 31-8959: Microbial Growth Inhibition.

M. Test Report Ro 31-8959 Activated Sludge Respiration Inhibition Test.

N. Test Report Ro 31-8959: Aerobic Biodegradation in Water.

O. Test Report Ro 31-8959 Toxicity to the Freshwater Green Alga, Selenastrum capricornutum, under Static Test Conditions.

P. Calculation of Maximum Expected Environmental Concentrations (MEEC) and Expected Environmental Concentrations (EEC).

Q. Test Report Ro 31-8959: Determination Soil Adsorption/Desorption.

R. Curriculum Vitae.

APPENDIX A

THE SYNTHETIC FLOW CHART

RO 31-8959/008

(CONFIDENTIAL)

APPENDIX B

PHYSICAL - CHEMICAL PROPERTY DATA

AND

MATERIAL SAFETY DATA SHEET

(MSDS)

FOR

SAQUINAVIR MESYLATE

PHYSICAL PROPERTY DATA SUMMARY

SAQUINAVIR MESYLATE

1. Solubility:

   Water 2.2 mg/ml

   1 - Octanol 0.13 mg/ml

   50% Ethanol/Water 23.2 mg/ml

2. Dissociation Constant

   Pka_1 = 1.1 conjugated acids of the quinoline group

   Pka2 = 7.1 decahydroisoquinoline group

3. Partition Coefficients

Aqueous medium	log P
n-octanol/water, 3.0	0.75
n-octanol/aqueous buffers 4.5	2.24
n-octanol/aqueous buffers 6.9	4.01
n-octanol/aqueous buffers 9.4	4.30

4. Melting Point 244°C

5. PH = Approximately 5 in 1% aqueous suspension.

ROCHE LABORATORIES
a division of Hoffmann-La Roche Inc.
340 Kingsland Street
Nutley, NJ 07110-1199

Emergency: (201) 235-6650
Chemtrec: (800)-424-9300
Information: (800) 526-6367

MATERIAL SAFETY DATA SHEET

SECTION 1. CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

Material Name .....: Saquinavir mesylate
Inventory Code .....: 50536
RO # .....: 31-8959/003
Synonyms .....: N-tert-Butyl-decahydro-2-[2(R)-hydroxy-4-phenyl-3(S)-[[N-(2-quinolylcarbonyl)-L-asparaginyl]amino]butyl]-(4aS,8aS)-isoquinoline-3(S)-carboxamide methanesulfonate
TSCA Status .....: FDA Exemption - Not on Inventory; R&D Exemption - Not on Inventory.
Chemical Family ....: Peptide-mimetic class
Therapeutic Category: Antiviral agent (HIV proteinase inhibitor)
Formulations Used In: INVIRASE(TM)

SECTION 2. COMPOSITION/INFORMATION ON INGREDIENTS

Ingredient Name	CAS Number	Concentration %
Saquinavir mesylate		>=98.5

SECTION 3. HAZARDS IDENTIFICATION

EMERGENCY OVERVIEW
Physical State .....: Fine Powder.
Color .....: White to off-white

Severe dust explosion hazard.

POTENTIAL HEALTH EFFECTS
Relevant Routes of Exposure .....: Inhalation, Ingestion.
Target Organs .....: Central Nervous System.

Acute Effects
General .....: May cause central nervous system effects such as headache, dizziness, drowsiness, fatigue, and lack of muscular coordination.

Chronic Effects ....: No adverse effects known.

Carcinogenicity ....: Not listed by NTP, IARC, or OSHA.

SECTION 4. FIRST AID MEASURES

Inhalation .....: Remove to fresh air. If discomfort occurs or persists, get medical attention.

Skin Contact .....: Remove contaminated clothing and shoes. Flush skin with plenty of water. If irritation occurs or persists, get medical attention. Wash clothing and shoes before reuse.

Eye Contact .....: Immediately flush eyes with plenty of water. If irritation occurs or persists, get medical attention.

Ingestion .....: If large quantities of this material are swallowed, get medical attention immediately. Do not induce vomiting unless directed by medical personnel. Never give anything by mouth to an unconscious person.

SECTION 5. FIRE FIGHTING MEASURES

Flash Point .....: Not Applicable

Extinguishing Media : Water, Carbon Dioxide, Dry Chemical, Foam.

Unusual Fire and Explosion Hazards ..: Severe dust explosion hazard. Toxic emissions may be given off in a fire. See Decomposition Products in Section 10. Stability and Reactivity.

Fire Fighting Instructions .....: Wear NIOSH/MSHA approved positive pressure, self contained breathing apparatus and full protective turn out gear. Use caution in approaching fire. Use water to keep fire exposed containers cool.

ST number .....: 2, Hartmann Tube.

SECTION 6. ACCIDENTAL RELEASE MEASURES

Spill Clean Up Procedures .....: Use proper personal protective equipment and clothing specified in Section 8-Exposure Controls/Personal Protection. Shut off the source of the spill or leak if it is safe to do so. Shut off all electrical equipment if it is safe to do so. Eliminate possible ignition sources. Follow appropriate grounding procedures. Scoop or shovel spilled material into a suitable labeled open head drum. Secure the drum cover and move the container to a safe holding area. Mop or flush the area with water. Collect wash with a noncombustible absorbent material and transfer to labeled container for treatment and disposal. Check area for residual material and repeat clean up if detected.

Treatment and Disposal .....: Decontaminate equipment. Dispose of protective clothing with the spilled material. Dispose of in accordance with recommendations in Section 13 Disposal Considerations.

SECTION 6. ACCIDENTAL RELEASE MEASURES (Continued. . .)

Reporting
Requirements .....: The United States Environmental Protection Agency (USEPA) has not established a Reportable Quantity (RQ) for releases of this material. In New Jersey, report all releases which are likely to endanger the public health, harm the environment or cause a complaint to the NJDEPE Hotline (1-609-292-5360) and to local officials. State and local regulations vary and may impose additional reporting requirements.

SECTION 7. HANDLING AND STORAGE

Special Sensitivity : Heat. Do not heat above 170 degrees C.
Handling & Storage
Precautions .....: Do not generate dust or expose to ignition sources.
Ground and bond all transfer equipment.
Milling/mixing/drying should be performed in devices equipped with explosion relief or suppression systems or under inert conditions.
Avoid contact with eyes, skin and clothing.
Avoid breathing dust.
Use with adequate ventilation.
When handling, use proper personal protective equipment specified in section 8.
Wash thoroughly after handling.
Keep container tightly closed when not in use.
Store in a cool, dry area.

SECTION 8. EXPOSURE CONTROLS / PERSONAL PROTECTION

ENGINEERING CONTROLS
Ventilation .....: Local ventilation is recommended when using this material. Use in a lab hood.

PERSONAL PROTECTION
Respirator Type(s) .: Negative Pressure Air Purifying, Half Face, Toxic Dust/Mist/Fume High Efficiency Filter.
Conditions for Use .: Respiratory protection is recommended under excessively dusty conditions. For production operations, a supplied-air full facepiece respirator or supplied-air hood is recommended. OSHA considers effective engineering controls to be the primary means to control worker exposure. Respiratory protection should not substitute for feasible engineering controls. Whenever respiratory protection is used, a complete respirator program should be developed in accordance with OSHA Subpart I (29CFR1910.134) requirements.
Glove Materials ....: Any plastic or rubber glove.
Conditions for Use .: Gloves are required if there is a potential for skin contact.

SECTION 8. EXPOSURE CONTROLS / PERSONAL PROTECTION (Continued. . .)

Skin Protection ....: Use protective clothing (disposable coveralls, lab coats, etc.) in both production and laboratory areas. Consult the protective clothing manufacturer, supplier and/or industrial hygienist.
Eye Protection .....: Safety Glasses Required.

OTHER CONTROL MEASURES
Administrative Controls .....: Post the work area and limit access to authorized personnel only.
Additional Protective Measures : Work clothing should be removed in a changeroom on site and laundered professionally. Employees should shower and change into street clothes before leaving the facility. Provide safety showers and eyewash stations in the work area. Prevent the accumulation of dust in the work area by thorough periodic cleaning of the area.

EXPOSURE LIMITS
There are no exposure limits specified either for this material or for any of its ingredients.

SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES

Physical State .....: Fine Powder.
Color .....: White to off-white
Molecular Weight ...: 766.96
Chemical Formula ...: C38H50N6O5.CH4O3S (1:1)
Pure/Mixture .....: Pure.
Melting Point .....: 244 C
H2O Solubility .....: 2.20 g/l; Slightly Soluble (0.1-1% by weight).
Solubility - Other .: Ethanol

SECTION 10. STABILITY AND REACTIVITY

Stability .....: Normally stable but may become unstable at elevated temperatures or reacts with water, releasing some energy but not violently.
Conditions to Avoid : Temperatures >100C
Dust Accumulation
Airborne Dust
Sources of Ignition
Incompatibility - Materials to Avoid .: Unknown.
Decomposition Products .....: Carbon monoxide, carbon dioxide, oxides of nitrogen, oxides of sulfur
Decomposition Temperature .....: 220 C
Polymerization .....: No

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SECTION 10. STABILITY AND REACTIVITY (Continued. . .)

Conditions of
Polymerization .....: Will not occur.

SECTION 11. TOXICOLOGICAL INFORMATION

Saquinavir mesylate

Acute Oral, Single Dose, Rat: >5000 mg/kg
Summary: The oral LD50 is greater than 5,000 mg/kg body weight (limit test) under the study conditions utilized.

Irritation Skin, 4 hour, Rabbit
Summary: No skin irritation was observed in rabbits after being exposed for 4 hours to a 0.5 g aliquot of this material.

Mutagenicity
Summary: No evidence of mutagenicity was observed in the Ames assay with or without metabolic activation, the unscheduled DNA synthesis assay, the mouse micronucleus test, the mammalian cell gene mutation assay (V79/HGPRT) and the chromosomal aberration assay with or without metabolic activation.

Reproductive Oral, Rat
Summary: No adverse effects were observed in peri and post-natal studies in rats at oral doses up to 1,600 mg/kg/day under the study conditions utilized. Also, no adverse effects were observed in a fertility and general reproductive study in rats at oral doses up to 1,200 mg/kg/day under the study conditions utilized.

Teratogenicity Oral, Rabbit
Summary: No evidence of teratogenicity was observed in rabbits who were treated orally with doses up to 1,000 mg/kg/day during gestation days 7 through 18 under the study conditions utilized.

Teratogenicity Oral, Rat
Summary: No evidence of teratogenicity was observed in rats who were treated orally with doses up to 1,600 mg/kg/day during gestation days 6 through 15 under the study conditions utilized.

Sensitization Skin, Guinea Pig
Summary: No evidence of sensitization was observed in a Guinea Pig Maximization Test under the study conditions utilized.

SECTION 12. ECOLOGICAL INFORMATION

No ecological data available on this material.

SECTION 13. DISPOSAL CONSIDERATIONS

Disposal
Recommendations ....: This material is suitable for incineration. These recommendations are based on the product as shipped. Use, processing, alteration or contamination may affect these disposal recommendations. State, local or site restrictions affecting the available proper disposal options may vary.
RCRA Waste # .....: Not regulated under RCRA
Empty Containers ...: Empty containers must be triple rinsed prior to disposal, recycling, or reuse.

SECTION 14. TRANSPORTATION INFORMATION

Enforcement Agency .: US Dept. of Transportation
Country/Community ..: USA
Proper Ship. Name ..: Non-regulated

Enforcement Agency .: International Air Transport Association
Country/Community ..: International
Proper Ship. Name ..: Non-regulated

SECTION 15. REGULATORY INFORMATION

No regulatory information available on this material.

SECTION 16. OTHER INFORMATION

APPROVAL INFORMATION
Preparer .....: Annette Bucca-Janacek
Approver .....: Corporate Environmental & Safety Affairs
Approval Date .....: 08/01/94
Reason For Issue ...: New MSDS

The information presented on this MSDS is, to the best of our knowledge, accurate and reliable. It is provided in good faith without warranty or acceptance of any liability on the part of Hoffmann-LaRoche, Inc. It is the responsibility of the user to evaluate the relevance and completeness of this information for their application and to determine the safety, suitability and status under applicable regulations relating to this product or byproducts arising out of their process.

APPENDIX C

LIST OF CHEMICAL SUBSTANCES ALONG WITH

CAS NO. ASSOCIATED WITH

MANUFACTURE OF DRUG

SUBSTANCE AND COPIES OF AVAILABLE MSDSs

(CONFIDENTIAL)

APPENDIX D

LIST OF CHEMICAL SUBSTANCES ALONG WITH

CAS NO. ASSOCIATED WITH

MANUFACTURE OF DRUG

PRODUCT AND COPIES OF AVAILABLE MSDSs

(CONFIDENTIAL)

APPENDIX E

THE MAXIMUM PROJECTED ANNUAL PRODUCTION

AND

ENERGY UTILIZATION DATA

(CONFIDENTIAL)

APPENDIX F

COMPLIANCE STATEMENTS SIGNED

BY A

HIGH RANKING OFFICIAL

FROM

VARIOUS COMPANIES

GENERAL COMPLIANCE STATEMENT

FIS - FABBRICA ITALIANA SINTETICI SPA

states

that it is in compliance with, or on an enforceable schedule to be in compliance with, all emission requirements set forth in permits, consent decrees and administrative orders applicable to the production of Nisylate (Ro 47-0950) at its facilities in Alte di Montecchio Maggiore - Vicenza, Italy as well as emission requirements set forth in applicable national and local statutes and regulations applicable to the production of Nisylate (Ro 47-0950) at its facilities in Alte di Montecchio Maggiore - Vicenza, Italy.

FIS - Fabbrica Italiana Sintetici Spa
Ing. M. Barcaro
Technical Manager

General Compliance Statement

Bayer AG states that it is in compliance with, or on an enforceable schedule to be in compliance with, all emission requirements set forth in permits, consent decrees and administrative orders applicable to the production of Ro 47-0950 at its facilities in Leverkusen and Dormagen as well as emission requirements set forth in applicable federal, state, and local statutes and regulations applicable to the production of Ro 47-0950 at its facilities in Germany.

Leverkusen, 14.12.1994

(Dr. Pelster)
Head of Chemical Production

Organic Chem. Business Group

ppa. Finzenhagen

(Dr. Finzenhagen)
Head of department
Environmental
Protection and Safety
Organic Chem. Business Group

General Compliance Statement

Orgamol SA states that it is in compliance with, or on an enforceable schecule to be in compliance with, all emission requirements set forth in permits, consent decrees and administrative orders applicable to the production of FT-Nisylate at its facilities in Evionnaz, Switzerland as well as emission requiements set forth in applicable federal, state, and local statutes and regulations applicable to the production of FT-Nisylate at its facilities in Evionnaz, Switzerland.

C. Rossi

Head of department
Environmental Protection

J.-P. Surbeck

Head of Pharma Operations
Manufacturing

ARCHIMICA S.p.A states that it is in compliance with, or on an enforceable schedule to be in compliance with, all emission requirements set forth in permits, consent decrees and administrative orders applicable to the production of Ro 31-9373 at its facilities in Origgio (VA), Italy as well as emission requirements set forth in applicable federal, state, and local statutes and regulations applicable to the production of Ro 31-9373 at its facilities in Origgio (VA), Italy.

ARCHIMICA S.p.A.
Managing Director
(Dr. Pietro Bellani)

GENERAL COMPLIANCE STATEMENT

Propeptide states that it is in compliance with, or on an enforceable schedule to be in compliance with, all emission requirements set forth in permits, consent decrees and administrative orders applicable to the production of Ro 31-9440 at its facilities in Vert le Petit France as well as emission requirements set forth in applicable state and local statutes and regulations applicable to the production of Ro 31-9440 at its facilities in Vert le Petit France

B. Cau

President of the Propeptide Company

GENERAL COMPLIANCE STATEMENT

Procos S.p.A. states that it is in compliance with, or on an enforceable schedule to be in compliance with, all emission requirements set forth in permits, consent decrees and administrative orders applicable to the production of Ro 31-9373 at its facilities in Cameri (NO) - Italy as well as emission requirements set forth in applicable state and local statutes and regulations applicable to the production of Ro 31-9373 at its facilities in Cameri (NO) - Italy.

Technical Director

Dr. Augusto Lavacchielli

Chemical Production

Dr. Augusto Menconi

Quality Control

Dr. Giovanni Colli

Micron Mills
A Subsidiary of Cowman Limited
Micron Mills Limited
8 Leesons Hill St Pauls Cray Orpington Kent BR5 2NH England
Tel (0689) 877095 Fax (0689) 891895

GENERAL COMPLIANCE STATEMENT

Micron Mills Limited states that it is in compliance with, or on an enforceable schedule to be in compliance with, all emission requirements set forth in consent decrees, and national and local statutes applicable to the milling of Saquinavir (Ro 31-8959) at its facilities in Orpington, England.

R.I. FUNNELL
Director of Operations

APPENDIX G

COMPLIANCE STATEMENTS

FROM

FOREIGN REGULATORY AUTHORITIES

11 MAR 1992

Regierungspräsidium Freiburg · Abholfach · 7800 Freiburg i. Br.

CU Chemie Uetikon GmbH

Raiffeisenstraße 4

D-7630 Lahr

Landeszentralbank Freiburg
Postgiroamt Karlsruhe
Landesgirokasse Filiale Freiburg

Staatsbauamt
Telex
Telefax
Telefax
Telefax

Freiburg i. Br.

Unser Zeichen

Unser Aktenzeichen
72/8823.12-009/A

2347 10.03.1992

Genehmigungsbehörde Nr.
Bauamt (örtlich)

3

(Bitte bei Antwort angeben)

Betreff:
Execution of the Federal Immission Control Act:
(BImSchG = Bumdes-Immissionsschutzgesetz)

Dear Sirs,

The Government of Freiburg as competent approving authority for the administrative district of Freiburg herewith certifies for presentation purposes at the American Food and Drug Administration (FDA) that the below mentioned facilities of

CU Chemie Uetikon GmbH, Raiffeisenstraße 4, D-7630 Lahr

are in accordance with the Federal Immission Control Act (BImSchG).

1.
Approval for erection and operation of a plant for process development and pilot production
Approval date: 18.04.1978

2.
Approval for erection and operation of a storehouse
Approval date: 11.12.1979

3.
Approval for erection and operation of a pilot plant with off-sites
Approval date: 29.06.1982

4.
Approval for erection and operation of a storehouse for organic and inorganic chemicals
Approval date: 17.02.1986

Dienstgebäude
Bürozeiten
Bankverbindung
Postscheckkonto
Briefanschrift

5.
Approval for extension of the pilot plant with extension of the storage facilities
Approval daie: 13.08.1987

6.
Approval for erection and operation of a CO_2-device
Approval date: 15.01.1990

7.
Approval for erection and operation of two storage tanks for storing of oxidizing agents and highly inflammable materials
Approval date: 12.02.1990

8.
Approval for erection and operation of eight immovable storage tanks for solvents and VbF-liquids (VbF = Regulations concerning burnable liquids)
Approval date: 02.08.1991

All the above approvals include requirements, e. g. to the emissions in the air and occupational health and safety standards, the compliance of which will be subject to the control by the responsible authorities.

Moll

Offenburg, 10.06.1992

Amt für Vasservirtschaft
und Bodenschutz
7600 Offenburg
Tgb.Nr. 5829

Bestätigung

Hiermit wird bestätigt, daß für die Abvasserverhältnisse im Bereich der Chemieanlagen mit Nebeneinrichtungen der Firma CU Chemie Uetikon GmbH, D- 7630 Lahr, Raiffeisenstr. 4, hauptsächlich das Vasserhaushaltsgesetz vom 23.09.1986, das Landesvassergesetz in der Passung vom 01.07.1988 und die Anforderungen der vasserrechtlichen Genehmigung des Amtes für Umveltschutz, Landratsamt Ortenaukreis in Offenburg, zuletzt geändert mit Nachtrag vom 23.01.1991, Az. 701-700.72, sovie damit verbundene Behördenkontrollen maßgebend sind.

To whom it may concern

- Confirmation -

This is to certify, that the vaste-vater-treatment of the chemical plants and its respective facilities of CU Chemie Uetikon GmbH at D - 7630 Lahr, Raiffeisenstr. 4, are subject of the Federal Vater Act "Vasserhaushaltsgesetz" dated 09/23/86 and the Vater Act of Baden-Württemberg "Vassergesetz" dated 07/01/88.

Further, it exists an official approval of the specified plant by the Regional Office for Environmental Protection, Offenburg, who issued the latest supplement to the respective approval on 01/23/91, reference no. 701.700.72.

Offenburg, 10.06.1992

Amt für Vasservirtschaft
und Bodenschutz
7600 Offenburg

Frei

Ihre Zeichen	Ihre Nachricht vom	Unsere Zeichen	Hausruf	Telefax	Datum
		IC-FE-Ol			02.09.1994

"Environmental Assessment"
(Umweltbetrachtung)
4-Nitrobenzolsulfonsäure
(4S,5S)-4-benzyl-2-oxooxazolidin-5-ylmethylester
(Nisylat)

Die Degussa AG als Hersteller von Nisylat mit der offiziellen Anschrift

Degussa AG
Weißfrauenstr. 9
60311 Frankfurt am Main

bestätigt hiermit, daß die Herstellung dieses Wirkstoffes und Zubereitungen dieses Wirkstoffes im

Werk Hanau Wolfgang
Rodenbach Chaussee 1
63403 Hanau

in einer nach den gültigen Gesetzen und Verordnungen der Bundesrepublik Deutschland zum Schutze der Umwelt genehmigten Anlage erfolgt.

Die Errichtung und das Betreiben von Anlagen zur Herstellung von pharmazeutischen Wirkstoffen ist im Sinne von § 4 des Bundesimmissionsschutzgesetzes (BImSchG) grundsätzlich genehmigungsbedürftig. Dies ergibt sich durch die Anwendung der Verordnungen über genehmigungsbedürftige Anlagen (4. BImSchV), in deren Anhang unter Ziffer 4.1 g

> Anlagen zur fabrikmäßigen Herstellung von Stoffen durch chemische Umwandlung insbesondere zur Herstellung von organischen Chemikalien ...

aufgeführt sind.

Für diese Anlagen ist ein schriftlicher Antrag zur Erteilung einer Genehmigung bei der zuständigen Genehmigungsbehörde zu stellen. Dazu bedarf es ausführlicher Unterlagen. Erst nach gründlicher Prüfung wird von der genehmigenden Behörde (im Falle der Degussa AG vom Regierungspräsidium Darmstadt) ein Genehmigungsbescheid erteilt. Dieser Bescheid darf

nur erteilt werden, wenn nach § 6 BImSchG (s. Anlage) bestimmte Genehmigungsvoraussetzungen erfüllt sind.

So muß unter anderem sichergestellt sein, daß die sich aus § 5 BImSchG ergebenden Pflichten des Betreibers genehmigungsbedürftiger Anlagen erfüllt werden. Außerdem dürfen andere öffentlich rechtliche Vorschriften sowie Belange des Arbeitsschutzes dem Errichten und dem Betreiben der Anlagen nicht entgegen stehen.

Nach § 5 BImSchG sind genehmigungsbedürftige Anlagen so zu errichten und zu betreiben, daß

1. Schädliche Umwelteinwirkungen und sonstige Gefahren, erhebliche Nachteile und erhebliche Belästigungen für die Allgemeinheit und die Nachbarschaft nicht hervorgerufen werden können,

2. Vorsorge gegen schädliche Umwelteinwirkungen getroffen wird, insbesondere durch die dem Stand der Technik entsprechenden Maßnahmen zur Emissionsbegrenzung,

3. Reststoffe vermieden werden, es sei denn, sie werden ordnungsgemäß und schadlos verwertet oder, soweit Vermeidung und Verwertung technisch nicht möglich oder zumutbar sind, als Abfälle ohne Beeinträchtigung des Wohls der Allgemeinheit beseitigt, und

4. entstehende Wärme für Anlagen des Betreibers genutzt oder an Dritte, die sich zur Abnahme bereit erklärt haben, abgegeben wird, soweit dies nach Art und Standort der Anlagen technisch möglich und zumutbar sowie mit den Pflichten nach Nummern 1 bis 3 vereinbar ist.

Die Einhaltung dieser beschriebenen gesetzlichen Regelungen wird von uns in Zusammenarbeit mit den zuständigen Behörden strikt befolgt. Vorsätzliche Abweichungen von diesen Vorschriften stellen Ordnungswidrigkeiten dar, die mit Geldbußen geahndet werden.

Im übrigen weisen wir darauf hin, daß wir der ständigen Aufsicht der staatlichen Überwachungsbehörden (SAIS, AIAS) unterliegen.

Hochachtungsvoll

Degussa Aktiengesellschaft

ppa. Siebert i.V. Dr. Sator

Dr. Siebert
Anlagen

Die Richtigkeit der vorstehenden Angaben wird hiermit bestätigt.

Darmstadt, 10 Oktober 1994

Regierungspräsident Darmstadt

REGIERUNGSPRÄSIDIUM DARMSTADT

i. A. Dostert

(Dr. Dostert)

§ 6

Genehmigungsvoraussetzungen

Die Genehmigung ist zu erteilen, wenn

1. sichergestellt ist, daß die sich aus § 5 und einer auf Grund des § 7 erlassenen Rechtsverordnung ergebenden Pflichten erfüllt werden, und
2. andere öffentlich-rechtliche Vorschriften und Belange des Arbeitsschutzes der Errichtung und dem Betrieb der Anlage nicht entgegenstehen.

Ihre Zeichen	Ihre Nachricht vom	Unsere Zeichen	Hausruf	Telefax	Datum
		IC-FE-O/			02.09.1994

"Environmental Assessment"
4-Nitrobenzenesulfonic acid
(4S,5S)-4-benzyl-2oxo-oxazolidin-5-ylmethyl ester
(Nisylate)

Degussa AG, as the manufacturer of Nisylate having of the offical address:

Degussa AG
Weißfrauenstr. 9
60311 Frankfurt am Main
Federal Republic of Germany

herewith certifies that the manufacture of Nisylate in

Site Hanau-Wolfgang
Rodenbach Chaussee 1
63403 Hanau
Federal Republic of Germany

is carried out in a plant according to the current laws and regulations of the Federal Republic of Germany for purposes of environmental protection.

The construction and the operation of plants for the manufacture of pharmaceutical products is principally subject to approval according to § 4 of the Bundesimmissionsschutzgesetz (BImSchG) (Federal Law on Immissions). This requirement is based on the regulations concerning plants subject to approval (4th BImSchV= Regulations on the Federal Law on Immissions), which are given in the enclosures under subparagraph 4.1g

> Plants for the manufacture of products by chemical conversion especially for the manufacture of organic chemicals ...

Approval for these plants is to be obtained by submitting a detailed written application to the relevant authorizing body. Only after through inspection is a notice of approval conferred by the authorizing body (for Degussa AG, plant Hanau-Wolfgang, Regierungspräsidium Darmstadt) (Directorate of the Administrative District, Darmstadt).

This notice may only be conferred if the relevant permit requirements have been satisfied according to § 6 BImSchG (see enclosure)

It is therefore essential to guarantee the fulfillment of the obligations in § 5 BImSchG which are incumbent on the operator of plants subject to approval. In addition, the construction and operation of the plants should not contravene further regulations under public law and the interests of industrial safety.

In accordance with § 5 BImSchG plants subjects to approval are to be constructed and operated in such a manner that

1. harmful environmental effects and other hazards, considerable disturbance and considerable inconvenience to the comunity and vicinity can not be evoked,

2. provisions are made against harmful environmental effects, in particular through the use of state-of-the-art measures for the limitation of emissions,

3. residues are avoided where possible, or are processed and rendered harmless according to regulations, or, where avoidance and processing is not technically feasible or is unreasonable, they are removed as waste without harm to well-being of the community, and

4. and resulting heat is used in the operator's plant or transferred to a third party that has declared itself as willing to accept, provided that the process involved is technically possible and acceptable, and that it is compatible with the obligations according to subparagraphs 1 to 3.

We will enforce strict measures, in cooperation with the authorities responsible, in order to ensure compliance with the legal rules described herein. Intentional violation of these regulations constitutes a legal irregularity which is punishable by fines.

Additionally, we draw attention to the fact that we are subject to the constant supervision of the public Industrial Inspection Board (SAIS, AIAS)

Yours faithfully

Degussa Aktiengesellschaft

ppa. Dr. Siebert

i.V. Dr Sator

Endosures

The accurancy of the given details is hereby confirmed.

Darmstadt 10. Oktober 1994

Regierungspräsident Darmstadt

i.A. Dostert

(Dr. Dostert)

REGIERUNGSPRÄSIDIUM DARMSTADT

Repubblica e Cantone
del Ticino

Il Dipartimento del territorio

CERTIFICATO DI PROTEZIONE AMBIENTALE

ENVIRONMENTAL PROTECTION CERTIFICATION

La Divisione dell'ambiente del Dipartimento del territorio certifica che l'attività produttiva della ditta

MICRO-MACINAZIONE SA
CH- 6995 MOLINAZZO DI
MONTEGGIO
SWITZERLAND

viene svolta nel pieno rispetto della normativa vigente in materia di protezione ambientale.

I relativi permessi coprono pure i processi di micronizzazione del principio attivo SAQUINAVIR (RD 31-8959)

I controlli periodici, eseguiti dalle istanze cantonali competenti, non hanno evidenziato situazioni non conformi alla citata legislazione

The Department for environmental protection ("Dipartimento del territorio") confirms that the company

MICRO-MACINAZIONE SA
CH- 6995 MOLINAZZO DI
MONTEGGIO
SWITZERLAND

has all the permits required by both federal and cantonal Swiss environmental protection laws and regulations to operate plants for the production of pharmaceutical substances.

The above mentioned permits cover also the micronisation of the active principle SAQUINAVIR (RD 31-8959).

During our regular inspections of the plants it has always be ascertained a completely satisfactory environmental situation.

./.

La Divisione dell'ambiente del Dipartimento del territorio è in particolare responsabile per l'applicazione delle seguenti leggi:

The Department for environmental protection is the authority, that is competent on the territory of the canton Ticino to enforce particularly the following federal and cantonal laws and regulations:

- Legge federale sulla protezione dell'ambiente del 07.10.1983
  Federal Law on the protection of the environment
- Ordinanza contro l'inquinamento atmosferico del 16.12.1985
  Air pollution control regulation
- Ordinanza sulle sostanze pericolose per l'ambiente del 09.06.1986
  Regulation on the containment of environmentally hazardous substances
- Ordinanza sul traffico dei rifiuti speciali del 12.11.1986
  Regulation on the handling of hazardous waste
- Ordinanza tecnica sui rifiuti del 10.12.1990
  Technical waste-matter regulation
- Ordinanza contro l'inquinamento fonico del 15.12.1986
  Noise protection regulation
- Ordinanza sull'esame di impatto ambientale del 19.10.1988
  Regulation on environmental compatibility audits
- Legge federale contro l'inquinamento delle acque del 24.01.1991
  Federal law on water protection
- Ordinanza sulle immissioni delle acque di rifiuto del 08.12.1975
  Regulation on the introduction of waste-water into the public sewage system
- Ordinanza generale sulla protezione delle acque del 19.06.1972
  General regulation on water protection
- Ordinanza sulla classificazione dei liquidi nocivi alle acque del 28.09.1981
  Regulation on the protection of water against water endangering substances
- Prescrizioni tecniche sui depositi liquidi del 21.06.1990
  Regulation on equipment for the storage and reloading of water endangering substances

DIPARTIMENTO DEL TERRITORIO

Il Direttore della Divisione dell'ambiente:

arch. Marcello Bernardi

Il Capo Sezione protezione aria e acqua:

dott. Mario Camani

COMUNE DI ORIGGIO
Via Dante Alighieri, 15
PROVINCIA DI VARESE
C.A.P. 21040 - Cod. Fisc. e P. IVA 00322990128
Ufficio Tecnico 02/96730034
Vigili Urbani 02/96732097
Fax 02/96730182

Prot. N. 9408

U ... N. ... Div. ...

Risposta a nota del ...

OGGETTO:

IL SINDACO

VISTA la domanda in data 5.9.1994, pervenuta in data 6.9.1994 al prot. n. 9408, presentata dalla Archimica S.p.A. con sede legale a Varese in via Staurenghi n. 9 ed attività ad Origgio in viale Europa n. 5;

VISTI gli atti d'ufficio,

CERTIFICA

che la Archimica S.p.A. è autorizzata, in conformità alle norme vigenti in materia, alla produzione dell'intermedio per uso farmaceutico denominato Ro-9373.
Si rilascia la presente certificazione in carta resa legale su richiesta dell'interessato per gli usi consentiti dalla Legge.

Origgio, 13 settembre 1994

IL SINDACO
(Dr. Giosuè Meazza)

COUNCIL OF THE ORIGGIO
Via Dante Alighieri 15
PROVINCE OF VARESE
Post Code 21040 - V.A.T. No. IT 00322990128

Phone: Secretary Office 02/96730726
Technical Office 02/96730034
Policemen 02/96732097
Fax 02/96730182

Reg. No. 2407 Date .....

Answer Date..... ..... No..... .....DN.....

SUBJECT:
STAMP - COUNCIL OF THE ORIGGIO - (Varese)

THE MAYOR

In consideration of the application dated September 5, 1994, we received on September 6, 1994 Reg. No. 9408, presented by Archimica S.p.A. with registered office at Varese via Staurenghi No. 9 and facility located at Origgio (VA) Viale Europa No. 5;

In consideration of the documentation showed,

CERTIFY

that Archimica S.p.A. is authorized, in compliance with the rules governing the matter, to produce the intermediate for pharmaceutical use denominated Ro 31-9373.
This certification has been issued on stamped paper on request of the applicant for all purposes allowed by law.

Origgio, September 13, 1994

THE MAYOR
(Dr. Giosuè Meazza)

STAMP - COUNCIL OF THE ORIGGIO (Varese)

We declare that the translation is accurate and is at the best level of our knowledge.

LE DÉPARTEMENT DE L'ÉCONOMIE PUBLIQUE

DAS VOLKSWIRTSCHAFTSDEPARTEMENT

102

- Vu l'art. 7, al. 2) de la loi fédérale sur le travail dans l'industrie, l'artisanat et le commerce, du 13 mars 1964 ;
- Vu l'art. 9, al. 1) de la loi cantonale sur le travail du 16 novembre 1966 ;
- Vu le préavis de l'Inspection fédérale du travail à Lausanne;
- Vu le préavis de la Caisse nationale suisse d'assurances en cas d'accident à Lausanne;
- Vu le préavis de l'Inspection cantonale du travail (Service social de protection des travailleurs et des relations du travail) à Sion ;

a u t o r i s e :

ORGAMOL SA, fabrication de produits chimiques, 1902 Evionnaz, à exploiter l'agrandissement de la halle K, dont les plans ont été approuvés en date du 9 mai 1989

Pour protéger la vie et la santé des travailleurs, l'employeur est tenu de prendre toutes les mesures dont l'expérience a démontré la nécessité, que l'état de la technique permet d'appliquer et qui sont adaptées aux conditions d'exploitation de l'entreprise.

Sion, le 5 novembre 1990

Droit de sceau : Fr. 50.--

Droits de timbre acquittés Caisse d'Etat du Valais

LE CHEF DU DEPARTEMENT DE L'ECONOMIE PUBLIQUE

Raymond Deferr, Conseiller d'Etat

Copie :

- IFT, Lausanne
- CNA, Lausanne
- Commission communale de surveillance d'Evionnaz
- Police cantonale de St-Maurice, p.v.d.s.

THE NATIONAL ECONOMY DEPARTMENT
DAS VOLKSWIRTSHAFTSDEPARTEMENT

- Pursuant to Section 7, Paragraph 2 of March 13 1964 Federal Law on Labor related to industry, craft industry and trade;

- Pursuant to Section 9, Paragraph 1 of November 16 1966 Cantonal Law on Labor;

- Pursuant to the Labor Federal Inspection's Notice in Lausanne;

- Pursuant to the Notice of the Swiss Caisse Nationale d'Assurances in case of an accident in Lausanne;

- Pursuant to the Labor Cantonal Inspection's Notice (Social Service of the workers' protection and of labor relations) in Sion;

AUTHORIZES :

ORGAMOL, Inc., manufacturing chemical products, 1902 Evionnaz, to set up a system in order to remove the storage of carbonyl chloride for which the plans have been approved on October 14 1987.

In order to protect the life and health of the workers, the employer must use all safety precautions which experience has demonstrated necessary, which can be applied given the present status of technology and are relevant to the running conditions of the company.

Dated: Sion, Switzerland, November 5, 1990

Seal duties. Fr. 50.

Stamp duties paid
to
Caisse d'Etat du Valais

by. THE NATIONAL ECONOMY DEPARTMENT OFFICER

Raymond Deferr, State Councillor

Copies to :

- IFT (Federal Inspection of Labor) Lausanne
- CNA (Caisse Nationale d'Assurances), Lausanne
- Evionnaz Local Monitoring Committee
- St-Maurice Cantonal Police

LE DÉPARTEMENT DE L'ECONOMIE PUBLIQUE

DAS VOLKSWIRTSCHAFTSDEPARTEMENT

- Vu l'art. 7, al. 2) de la loi fédérale sur le travail dans l'industrie, l'artisanat et le commerce, du 13 mars 1964 ;

- Vu l'art. 9, al. 1) de la loi cantonale sur le travail du 16 novembre 1966 ;

- Vu le préavis de l'Inspection fédérale du travail à Lausanne du 13 avril 1989;

- Vu le préavis de l'Inspection cantonale du travail (Service social de protection des travailleurs et des relations du travail) à Sion ;

a u t o r i s e :

ORGAMOL SA, produits chimiques, 1902 EVIONNAZ à exploiter la modification de l'usine 6 / 2ème projet (plans approuvés le 23 décembre 1986, autorisation d'exploiter provisoire délivrée le 31 août 1987).

1. Pour protéger la vie et la santé des travailleurs, l'employeur est tenu de prendre toutes les mesures dont l'expérience a démontré la nécessité, que l'état de la technique permet d'appliquer et qui sont adaptées aux conditions d'exploitation de l'entreprise.

Sion, le 18 avril 1989

Droit de sceau : Fr. 20.--

Caisse d'Etat du Valais

LE CHEF DU DEPARTEMENT DE L'ECONOMIE PUBLIQUE

Raymond Deferr, Conseiller d'Etat

Copie :

- IFT, Lausanne
- CNA, Lausanne
- Commission communale de surveillance de Evionnaz
- Police cantonale de St-Maurice, p.v.d.s.

THE NATIONAL ECONOMY DEPARTMENT
DAS VOLKSWIRTSHAFTSDEPARTEMENT

- Pursuant to Section 7, Paragraph 2 of March 13 1964 Federal Law on Labor related to industry, craft industry and trade;

- Pursuant to Section 9, Paragraph 1 of November 16 1966 Cantonal Law on Labor;

- Pursuant to the Labor Federal Inspection's Notice in Lausanne;

- Pursuant to the Labor Cantonal Inspection's Notice (Social Service of the workers' protection and of labor relations) in Sion.

AUTHORIZES :

ORGAMOL, Inc., chemical products, 1902 Evionnaz, to apply modifications to the plant No. 6 / 2nd project (for which the plans were approved on December 23 1986; temporary authorization to modify was given on August 31 1987).

In order to protect the life and health of the workers, the employer must use all safety precautions which experience has demonstrated necessary, which can be applied given the present status of technology and are relevant to the running conditions of the company.

Dated: Sion, Switzerland, April 18 1989

Seal duties: Fr. 20.

Stamp duties paid
to
Caisse d'Etat du Valais

by: THE NATIONAL ECONOMY DEPARTMENT OFFICER

Raymond Defer, State Councillor

Copies to :

- IFT (Federal Inspection of Labor), Lausanne
- CNA (Caisse Nationale d'Assurances), Lausanne
- Evionaz Local Monitoring Committee
- St Maurice Cantonal Police

LE DÉPARTEMENT DE L'ÉCONOMIE PUBLIQUE
DAS VOLKSWIRTSCHAFTSDEPARTEMENT

- Vu l'art. 7, al. 2) de la loi fédérale sur le travail dans l'industrie, l'artisanat et le commerce, du 13 mars 1964 ;
- Vu l'art. 9, al. 1) de la loi cantonale sur le travail du 16 novembre 1966 ;
- Vu le préavis de l'Inspection fédérale du travail à Lausanne;
- Vu le préavis de la Caisse nationale suisse d'assurances en cas d'accident à Lausanne;
- Vu le préavis de l'Inspection cantonale du travail (Service social de protection des travailleurs et des relations du travail) à Sion ;

a u t o r i s e :

ORGAMOL SA, fabrication de produits chimiques, 1902 Evionnaz, à exploiter l'installation d'élimination du stockage de chlorure de carbonyle, dont les plans ont été approuvés en date du 14 octobre 1987

Pour protéger la vie et la santé des travailleurs, l'employeur est tenu de prendre toutes les mesures dont l'expérience a démontré la nécessité, que l'état de la technique permet d'appliquer et qui sont adaptées aux conditions d'exploitation de l'entreprise.

Sion, le 5 novembre 1990

Droit de sceau : Fr. 50.--

Droits de timbre acquittés
Caisse d'Etat du Valais

LE CHEF DU DEPARTEMENT DE L'ECONOMIE PUBLIQUE

Raymond Deferr, Conseiller d'Etat

Copie :

- IFT, Lausanne
- CNA, Lausanne
- Commission communale de surveillance d'Evionnaz
- Police cantonale de St-Maurice, p.v.d.s.

THE NATIONAL ECONOMY DEPARTMENT
DAS VOLKSWIRTSHAFTSDEPARTEMENT

- Pursuant to Section 7, Paragraph 2 of March 13 1964 Federal Law on Labor related to industry, craft industry and trade;

- Pursuant to Section 9, Paragraph 1 of November 16 1966 Cantonal Law on Labor;

- Pursuant to the Labor Federal Inspection's Notice in Lausanne;

- Pursuant to the Notice of the Swiss Caisse Nationale d'Assurances in case of an accident in Lausanne;

- Pursuant to the Labor Cantonal Inspection's Notice (Social Service of the workers' protection and of labor relations) in Sion;

AUTHORIZES :

ORGAMOL, Inc., manufacturing chemical products, 1902 Evionnaz, to run the expansion of the Market K, for which the plans have been approved on May 9 1989.

In order to protect the life and health of the workers, the employer must use all safety precautions which experience has demonstrated necessary, which can be applied given the present status of technology and are relevant to the running conditions of the company.

Dated: Sion, Switzerland, November 5, 1990

Seal duties: Fr. 50.

Stamp duties paid
to
Caisse d'Etat du Valais

by: THE NATIONAL ECONOMY DEPARTMENT OFFICER

Raymond Deferr, State Councillor

Copies to :

- IFT (Federal Inspection of Labor), Lausanne
- CNA (Caisse Nationale d'Assurances), Lausanne
- Evionnaz Local Monitoring Committee
- St-Maurice Cantonal Police

ENVIRONMENTAL PROTECTION CERTIFICATE

1. The company F. HOFFMANN-LA ROCHE LTD operates facilities for chemical and pharmaceutical manufactunng at the following address:

F. HOFFMANN-LA ROCHE Ltd.
Grenzacherstrasse 124
CH-4002 Basel
Switzerland

2. These production facilities may only operate in accordance with permits issued by the responsible Authorities. In the permits are laid down the purpose for witch buildings and plants may be used and the legal conditions with which the Company must comply.

3. The above-described permits also cover the preparation of the Active Substance

Saquinavir

and the Pharmaceutical Preparation

INVIRASE Capsules

4. All buildings and plants of the company F. Hoffmann-La Roche Ltd. must comply with the federal and cantonal laws and regulations concerning safety, protection of the environment and working conditions.

5. The relevant departments of the Cantonal Authorities perform periodic inspections.

6. It can here be stated that the undersigned Governmental Office has proved the correct building and producing permits are given.

Basel, 7. April 1995/pg

BAUDE...
Der D...

Dr. G. Vischer

MÜNSTERPLATZ 11 · CH-4001 BASEL TELEFON 061 267 60 91 · TELEFAX 061 267 94 50

Repubblica e Cantone
del Ticino

Il Dipartimento del territorio

CERTIFICATO DI PROTEZIONE AMBIENTALE

ENVIRONMENTAL PROTECTION CERTIFICATION

La Divisione dell'ambiente del Dipartimento del territorio certifica che l'attività produttiva della ditta

MICRO-MACINAZIONE SA
CH- 6995 MOLINAZZO DI
MONTEGGIO
SWITZERLAND

viene svolta nel pieno rispetto della normativa vigente in materia di protezione ambientale.

I relativi permessi coprono pure i processi di micronizzazione del principio attivo SAQUINAVIR (RD 31-8959)

I controlli periodici, eseguiti dalle istanze cantonali competenti, non hanno evidenziato situazioni non conformi alla citata legislazione.

The Department for environmental protection ("Dipartimento del territorio") confirms that the company

MICRO MACINAZIONE SA
CH- 6995 MOLINAZZO DI
MONTEGGIO
SWITZERLAND

has all the permits required by both federal and cantonal Swiss environmental protection laws and regulations to operate plants for the production of pharmaceutical substances.

The above mentioned permits cover also the micronisation of the active principle SAQUINAVIR (RD 31-8959).

During our regular inspections of the plants it has always be ascertained a completely satisfactory environmental situation.

./.

La Divisione dell'ambiente del Dipartimento del territorio è in particolare responsabile per l'applicazione delle seguenti leggi:

The Department for environmental protection is the authority, that is competent on the territory of the canton Ticino to enforce particularly the following federal and cantonal laws and regulations:

- Legge federale sulla protezione dell'ambiente del 07.10.1983
  Federal Law on the protection of the environment
- Ordinanza contro l'inquinamento atmosferico del 16.12.1985
  Air pollution control regulation
- Ordinanza sulle sostanze pericolose per l'ambiente del 09.06.1986
  Regulation on the containment of environmentally hazardous substances
- Ordinanza sul traffico dei rifiuti speciali del 12.11.1986
  Regulation on the handling of hazardous waste
- Ordinanza tecnica sui rifiuti del 10.12.1990
  Technical waste-matter regulation
- Ordinanza contro l'inquinamento fonico del 15.12.1986
  Noise protection regulation
- Ordinanza sull'esame di impatto ambientale del 19.10.1988
  Regulation on environmental compatibility audits
- Legge federale contro l'inquinamento delle acque del 24.01.1991
  Federal law on water protection
- Ordinanza sulle immissioni delle acque di rifiuto del 08.12.1975
  Regulation on the introduction of waste-water into the public sewage system
- Ordinanza generale sulla protezione delle acque del 19.06.1972
  General regulation on water protection
- Ordinanza sulla classificazione dei liquidi nocivi alle acque del 28.09.1981
  Regulation on the protection of water against water endangering substances
- Prescrizioni tecniche sui depositi liquidi del 21.06.1990
  Regulation on equipment for the storage and reloading of water endangering substances

DIPARTIMENTO DEL TERRITORIO

Il Direttore della Divisione dell'ambiente:

arch. Marcello Bernardi

Il Capo Sezione protezione aria e acqua:

dott. Mario Camani

APPENDIX H

COPY OF INCINERATOR AND INDUSTRIAL LANDFILL PERMITS FOR
F. HOFFMANN-La ROCHE Ltd, HOFFMANN-La ROCHE Inc,
AND
CONTRACTORS

BAU- UND UMWELTSCHUTZ
KANTON BASEL-STADT
GEWÄSSERSCHUTZAMT

Rech.Nr.: 933008
Gebühr : Fr. 450.-
Budg.Kt.: 612.042.731.500

EINSCHREIBEN

F. Hoffmann-La Roche AG
Pharma Stammhaus
Sicherheit und Umweltschutz

4002 Basel

U/Z.: Eh 6.64

Basel, 22. Dezember 1993

VVS-EMPFÄNGERBEWILLIGUNG / VERFÜGUNG

Sehr geehrte Damen und Herren

Gestützt auf Art. 29 der Eidgenössischen Verordnung über den Verkehr mit Sonderabfällen (VVS) vom 12. November 1988 erteilen wir Ihnen eine Bewilligung zur Annahme, Zwischenlagerung und Behandlung bestimmter Sonderabfälle.

AUSSTELLUNGSDATUM	:	22. Dezember 1993
ABLAUFDATUM	:	31. Dezember 1998
BEWILLIGUNGSINHABER	:	F. Hoffmann-La Roche AG Pharma Stammhaus Sicherheit und Umweltschutz 4002 Basel
VERANTWORTLICH	:	Herr Dr. E. Kräuchi
BETRIEBSNUMMER	:	27010057
BEHANDLUNGSARTEN	:	Zwischenlagerung Verbrennung chemisch-physikalische Behandlung Recycling / Stripping
BEHANDLUNGSORTE	:	Bau 41, 47 → Zwischenlagerung Bau 35 → Verbrennung PSUU-Labors oder Anfallsort → chem.-phys. Behandlung Bau 29 → Recycling / Stripping ARA Ciba-Geigy / Roche
VVS-CODE / ABFALLART	:	siehe Beilage

HOCHBERGERSTRASSE 158 · CH-4019 BASEL
TELEFON 061 65 22 22 · TELEFAX 061 65 29 87
TELEX 96 53 17 gsbch

NEW JERSEY STATE DEPARTMENT

DIVISION OF ENVIRONMENTAL QUALITY
AIR POLLUTION CONTROL PROGRAM

All Correspondence must indicate your APC PLANT ID NUMBER

Certificate Number 113190 APC PLANT ID 30374

(Mailing Address)

HOFFMANN-LA ROCHE, INC. C/C ENVIR. AFFRS
340 KINGSLAND ST.
NUTLEY NJ 07110

(Plant Location)

RTE. 3
CLIFTON

Applicant's Designation of Equipment BUILDING 43
N.J. Stack No. 798 No. of Stacks 001 No. of Sources 01
Approval Effective 06/28/93 Expiration 06/27/95

* TEMPORARY CERTIFICATE TO OPERATE CONTROL APPARATUS OR EQUIPMENT *

* CONDITIONAL 90 DAY EXTENSION *

THIS TEMPORARY CERTIFICATE IS BEING EXTENDED TO ALLOW FOR FURTHER FIELD/OFFICE EVALUATION.

THIS EXTENSION SHALL NOT BE CONSTRUED TO EXTEND THE COMPLIANCE DATES(S) OF ANY ORDER ISSUED BY OR ENTERED INTO WITH THE DEPARTMENT AS THE RESULT OF AN ADMINISTRATIVE OR JUDICIAL ACTION.

THE EQUIPMENT COVERED BY THIS CERTIFICATE MAY BE SUBJECT TO PERIODIC COMPLIANCE INSPECTIONS, PURSUANT TO N.J.A.C. 7:27-8.8(C). YOU WILL BE INVOICED FOR A $200 FEE AFTER EACH PERIODIC COMPLIANCE INSPECTION. YOU WILL NOT BE INVOICED FOR AN INSPECTION DURING WHICH A 5 YEAR APPROVAL DETERMINATION IS MADE. YOU MAY BE INVOICED FOR FEES FOR OTHER SERVICES THAT ARE PERFORMED BY THE DEPARTMENT PURSUANT TO CONDITIONS OF APPROVAL.

IF THE DEPARTMENT IS SOLELY RESPONSIBLE FOR BEING UNABLE TO INSPECT THIS EQUIPMENT IN OPERATION AS PERMITTED DURING THIS 90-DAY PERIOD, THIS TEMPORARY CERTIFICATE WILL BE EXTENDED AUTOMATICALLY. HOWEVER, IF YOU ARE RESPONSIBLE FOR THE DEPARTMENT'S BEING UNABLE TO INSPECT, E.G., NOT NOTIFYING THE DEPARTMENT WHEN THIS EQUIPMENT OR PROCESS IS IN OPERATION, THIS CERTIFICATE MAY NOT BE EXTENDED AND YOU WILL BE NOTIFIED BY THE DEPARTMENT THAT YOU MUST APPLY FOR AND OBTAIN AN EXTENSION AUTHORIZING YOU TO CONTINUE TO OPERATE THE EQUIPMENT. THE DEPARTMENT RESERVES THE RIGHT TO WITHHOLD ANY EXTENSION OF THIS TEMPORARY CERTIFICATE, IN WHICH EVENT YOU WILL BE NOTIFIED THAT YOU MUST APPLY FOR AND OBTAIN AN EXTENSION AUTHORIZING YOU TO CONTINUE TO OPERATE AFTER THE EXPIRATION DATE OF THIS CERTIFICATE.

IN ACCORDANCE WITH N.J.A.C. 7:27-8.3(D), THIS CERTIFICATE MUST BE READILY AVAILABLE FOR INSPECTION ON THE OPERATING PREMISES.

PLEASE REFER TO YOUR INITIAL PERMIT APPROVAL FOR OPERATING CONDITIONS.

Approved by: Donald Patterson

NRO - PASSAIC COUNTY HEALTH DEPARTMENT 03/06/95-53

QUESTIONS CONCERNING THIS DOCUMENT SHOULD BE DIRECTED TO THE REGIONAL OFFICE INDICATED BELOW THAT COVERS THE COUNTY IN WHICH YOUR FACILITY IS LOCATED:

REGIONAL OFFICES

CENTRAL REGIONAL ENFORCEMENT
Burlington, Mercer, Middlesex, Monmouth, Ocean counties

New Jersey Department of Environmental Protection
Division of Environmental Quality
Bureau of Enforcement Operations
CN-407
Trenton, NJ 08625
(609)584-4100

METROPOLITAN REGIONAL ENFORCEMENT
Bergen, Essex, Hudson, Union counties

New Jersey Department of Environmental Protection
Division of Environmental Quality
Bureau of Enforcement Operations
3 Babcock Place
West Orange, NJ 07052
(201)669-2935

NORTHERN REGIONAL ENFORCEMENT
Hunterdon, Morris, Passaic, Somerset, Sussex, Warren counties

New Jersey Department of Environmental Protection
Division of Environmental Quality
Bureau of Enforcement Operations
1259 Route 46, Building 2
Parsippany, NJ 07054
(201)299-7700

SOUTHERN REGIONAL ENFORCEMENT
Atlantic, Camden, Cape May, Cumberland, Gloucester, Salem counties

New Jersey Department of Environmental Protection
Division of Environmental Quality
Bureau of Enforcement Operations
20 East Clementon Road, Suite 302N
Gibbsboro, NJ 08026
(609)346-8071

QUESTIONS CONCERNING THE FOLLOWING TOPICS SHOULD BE DIRECTED TO THE APPROPRIATE AGENCY OF THE NEW JERSEY DEPARTMENT OF ENVIRONMENTAL PROTECTION, DIVISION OF ENVIRONMENTAL QUALITY, INDICATED BELOW:

Questions concerning certificates covering gas stations, commercial dry cleaners and small spray booth operations, including auto body shops:	Questions concerning permits to construct and emissions banking:	Questions concerning stack testing and continuous emission monitor auditing:
Minor Source Compliance Investigation CN-407 Trenton, NJ 08625 (609)584-4240	Bureau of New Source Review CN-027, 401 East State Street Trenton, NJ 08625 (609)292-6716	Bureau of Technical Services CN-411 Trenton, NJ 08625 (609)530-4041

CATEGORY I includes the following types of equipment:

a) Gasoline vapor recovery systems at any retail gasoline facilities (not including such systems at bulk terminals);
b) Liquid or gaseous commercial fossil fuel (not including coal or other solid fuel, non-commercial fuel, crude oil or process by-products in any form) burning equipment having a designed heat input rate of less than 10 million BTU per hour;
c) Liquid (except any toxic substance or "TXS" listed in N.J.A.C. 7:27-17) stationary storage tanks with capacities of less than 20,000 gallons and any control apparatus serving such tanks;
d) Emergency diesel generators with less than 10 megawatts of electrical output that operate less than 500 hours per year;
e) Any control apparatus that solely serves one or more laboratory hoods or other equipment that exhausts fumes from laboratory operations;
f) The following types of equipment, when equipped with particulate control apparatus that achieves a minimum collection efficiency of 99%, and the control apparatus serving the equipment:
   1) Woodworking equipment;
   2) Metalworking equipment;
   3) Solid particle (except any TXS) storage vessels;
   4) Enclosed stationary solid material (except any TXS) handling equipment using pneumatic, bucket or belt conveying systems;
   5) Paint spray booths that use less than one half gallon of paint per hour, and that neither emit nor have the potential to emit any TXS.

CATEGORY II includes all types of equipment except those that belong to CATEGORY I.

State of New Jersey
Department of Environmental Protection and Energy
Division of Solid Waste Management
CN 414
Trenton, NJ 08625-0414
Tel # 609-530-8591
Fax # 609-530 8899

Scott A. Weiner
Commissioner

Steven Gabel
Director

ENVIRONMENTAL AND HEALTH IMPACT STATEMENT APPROVAL

Under the provisions of N.J.S.A. 13:1E-26, known as the Solid Waste Management Act, Environmental Impact Statement Approval is hereby issued to:

Hoffmann-LaRoche, Inc.

FOR THE PURPOSE OF CONSTRUCTING AND OPERATING A:	Small Scale Incinerator
ON LOT NO.(S):	a portion of 4
ON BLOCK NO.(S):	80.02
IN THE MUNICIPALITY OF:	Clifton
COUNTY:	Passaic

The Department has reviewed the Environmental Impact Statement and additional information pursuant to the Solid Waste Management Act, N.J.S.A. 13:1E-1 et seq., specifically, N.J.S.A. 13:1E-26, and the following findings are made:

1. The proposed construction and operation of the above named facility is consistent with the adopted and approved Passaic County District Solid Waste Management Plan.

2. The Small Scale Incinerator Facility will be constructed and operated pursuant to the standards adopted and promulgated by the Department pursuant to N.J.S.A. 13:1E-1 et seq.

This Approval shall become null and void upon the expiration or revocation of any Solid Waste Facility Permit issued by this Division to the particular solid waste facility herein identified or if full permit authorization has not been obtained for the above facility within three years of the issuance of this Approval.

The issuance of this document shall not be construed as authorization to operate a solid waste facility. Operations may commence only upon obtaining a Solid Waste Facility Permit issued by the Division of Solid Waste Management pursuant to N.J.S.A. 13:1E-1 et seq.

This Approval has been granted in conformance with the existing Rules and Regulations of the Division of Solid Waste Management. Should changes in these Rules become effective, the Department reserves the right to re-evaluate said Approval and require modifications as deemed appropriate.

JUN 30 1993

Date

S Bhalla

Sukhdev Bhalla, P.E.
Acting Assistant Director
Engineering Element

NEW YORK STATE DEPARTMENT OF ENVIRONMENTAL CONSERVATION

State Pollutant Discharge Elimination System (SPDES)

DISCHARGE PERMIT

Special Conditions (Part I)

117

Industrial Code: 4953
Discharge Class (CL): 03
Toxic Class (TX): T
Major Drainage Basin: 01
Sub Drainage Basin: 01
Water Index Number: O-158
Compact Area: IJC

SPDES Number: NY-0072061
DEC Number: 9-2934-00022/00049-0
Effective Date (EDP): 10/8/93
Expiration Date (EDP): 10/1/98
Modification Date(s):
Attachment(s): General Conditions (Part II) Date: 11/90

This SPDES permit is issued in compliance with Title 8 of Article 17 of the Environmental Conservation Law of New York State and in compliance with the Clean Water Act as amended, (33 U.S.C. Section 1251 et. seq.)(hereafter referred to as "the Act").

PERMITTEE NAME AND ADDRESS

Attention: Rodger Henson, CWM President

Name: CWM Chemical Services, Inc.
Street: 1550 Balmer Road
City: Model City State: NY Zip Code: 14107

is authorized to discharge from the facility described below:

FACILITY NAME AND ADDRESS

Name: CWM Chemical Services, Inc.
Location (C,T,V): Porter (T) County: Niagara
Facility Address: 1550 Balmer Road
City: Model City State: NY Zip Code: 14107
NYTM - E: 177 . 1 NYTM - N: 4 793 . 1
From Outfall No.: 001 at Latitude: 43° 13′ 06" & Longitude: 79° 02′ 56"
Into receiving waters known as: Niagara River Class: A-Special
and: (list other Outfalls, Receiving Waters & Water Classifications)
002, Tributary of Fourmile Creek, O-156-1c-3 Class C
003, Tributary of Fourmile Creek, O-156-1c-3 Class C

In accordance with the effluent limitations, monitoring requirements and other conditions set forth in Special Conditions (Part I) and General Conditions (Part II) of this permit.

DISCHARGE MONITORING REPORT (DMR) MAILING ADDRESS

Mailing Name: CWM Chemical Services, Inc.
Street: 1550 Balmer Road
City: Model City State: NY Zip Code: 14107
Responsible Official or Agent: Jill Knockerbocker, Env. Mgr. Phone: (716)754-8231

This permit and the authorization to discharge shall expire on midnight of the expiration date shown and the permittee shall not discharge after the expiration date unless this permit has been renewed, or extended pursuant to law. To be authorized to discharge beyond the expiration date, the permittee shall apply for a permit renewal no less than 180 days prior to the expiration date shown above.

DISTRIBUTION
DRA File No. 9-2934-00022/00049-0
Mr. R. Speed/Mr. D. Leemhuis
Mr. R. Hannaford - BWFD-TSS, Albany
EPA Region II; Mr. J. Devald - NCHD

Permit Administrator: Paul D. Eismann (Deputy)
Address: NYSDEC - Region 9
270 Michigan Ave., Buffalo, NY 14203-2999
Signature: [signature] Date: 10/8/93

COMMONWEALTH OF PENNSYLVANIA
DEPARTMENT OF ENVIRONMENTAL RESOURCES
1875 New Hope Street
Norristown, PA 19401
215 270-1920

May 31, 1985

Mr. Nolan Perin
Grand Central Sanitary Landfill
R.D. #1, Box 211
Pen Argyl, PA 18072

Re: Module #1 Submission
Asbestos Containing Waste

Dear Mr. Perin:

I am pleased to enclose Permit No. 100265 for the operation of your processing or disposal facility. It is issued in accordance with Act 97, the Pennsylvania Solid Waste Management Act.

Compliance with the limitations and stipulations that have been set forth on your permit is mandatory. You have the right to appeal any limitation or stipulation as stated on your permit.

This action of the Department may be appealable to the Environmental Hearing Board, Third Floor, 221 N. Second Street, Harrisburg, PA 17101, (717-787-3483) by any aggrieved person pursuant to Section 1921-A of the Administrative Code of 1929, 71 P.S. Section 510-21; and the Administrative Agency Law, 2 Pa. C.S., Chapter 5A. Appeals must be filed with the Environmental Hearing Board within 30 days of receipt of written notice of this action unless the appropriate statute provides a different time period. Copies of the appeal form and the regulations governing practice and procedure before the Board may be obtained from the Board. This paragraph does not, in and of itself, create any right of appeal beyond that permitted by applicable statutes and decisional law.

If you have any questions concerning the enclosed permit and/or the requirements set forth by the Pennsylvania Solid Waste Management Act, please contact the Bureau of Solid Waste Management, 1875 New Hope Street, Norristown, PA 19401, AC215-270-1920.

Sincerely yours,

Wayne L. Lynn

WAYNE L. LYNN
Regional Solid Waste Manager

cc: Plainfield Township
Lehigh-Northampton Joint Planning Commission
30 File
Re 44748.50

ENCLOSURE

,.Y 38 5/82

COMMONWEALTH OF PENNSYLVANIA
DEPARTMENT OF ENVIRONMENTAL RESOURCES
BUREAU OF SOLID WASTE MANAGEMENT

FORM NO. 12-A

MODIFICATION TO SOLID WASTE DISPOSAL AND/OR PROCESSING PERMIT

Under the provisions of Act 97, the Solid Waste Management Act of July 7, 1980, Solid Waste Permit Number 100265 issued on (date original permit was issued) 11/13/80 to (permittee.) Grand Central Sanitary Landfill, Inc.
(address) P. O. Box 211, R. D. #1, Pen Argyl, PA 18072

is hereby modified as follows:

1. This amended solid waste permit is issued based upon application No. 100265 which was received in the Norristown Regional Office of the Department of Environmental Resources on November 14, 1984. This amended solid waste permit is for the disposal of asbestos containing waste from demolition projects at the Grand Central Sanitary Landfill located in Plainfield Township, Northampton County. This approved application includes a satisfactory response to the Department's February 25, 1985 review letter.

2. Nothing in this permit shall be construed to supersede, amend or authorize violation of, the provisions of any valid and applicable local law, ordinance or regulation, provided that said local law, ordinance or regulation is not pre-empted by the Pennsylvania Solid Waste Management Act, the Act of July 7, 1980, Act 97, 35 P.S. 6018.101, et seq.

This modification shall be attached to the existing Solid Waste Permit described above and shall become a part thereof effective on (date) 5/31/85

Wayne D. Lynn
FOR THE DEPARTMENT OF ENVIRONMENTAL RESOURCES

APPENDIX I

SAQUINAVIR (RO 31-8959): TECHNICAL SUMMARY OF ABSORPTION AND DISPOSITION IN ANIMALS

(CONFIDENTIAL)

APPENDIX J

TEST REPORT RO 31-8959: ACUTE TOXICITY

TO THE

WATER FLEAS DAPHNIA MAGNA

UNDER STATIC CONDITIONS

(CONFIDENTIAL)

APPENDIX K

TEST REPORT RO 31-8959: ACUTE TOXICITY TO RAINBOW TROUT *ONCORHYNCHUS MYKISS*, UNDER STATIC CONDITIONS

(CONFIDENTIAL)

APPENDIX L

TEST REPORT RO 31-8959:

MICROBIAL GROWTH INHIBITION

(CONFIDENTIAL)

APPENDIX M

TEST REPORT RO 31-8959: ACTIVIATED SLUDGE RESPIRATION INHIBITION TEST

(CONFIDENTIAL)

APPENDIX N

TEST REPORT RO 31-8959:

AEROBIC BIODEGRADATION IN WATER

(CONFIDENTIAL)

APPENDIX Q

TEST REPORT RD 31-8959: TOXICITY TO

THE FRESHWATER GREEN ALGA,

SELENASTRUM CAPRICORNUTUM,

UNDER

STATIC TEST CONDITIONS

(CONFIDENTIAL)

APPENDIX P

CALCULATION OF MAXIMUM EXPECTED ENVIRONMENTAL CONCENTRATIONS (MEEC) AND EXPECTED ENVIRONMENTAL CONCENTRATIONS

(CONFIDENTIAL)

APPENDIX Ω

TEST REPORT RO 31-8959: DETERMINATION OF SOIL ADSORPTION/DESORPTION

(CONFIDENTIAL)

APPENDIX R

CURRICULUM VITAE

CURRICULUM VITAE

NAME: P. V. Shah

ADDRESS: 28 Onyx Court
Passaic, N.J. 07055

201-235-4212 (Office)
201-614-1065 (Home)

PERSONAL DATA: Date & Place of Birth: 2/17/49, Kamalchhod, Gujarat State, India.
Citizenship: U. S.

QUALIFICATIONS: Ph.D., M.S. and M.T. (ASCP)- Certified Medical Technologist

EDUCATION:

05/82	-	Ph.D. from N.C. State University, Raleigh, NC
10/74	-	Medical Technologist. Certified by American Society of Clinical Pathologists, Chicago, IL
08/73	-	Medical Technology, The Memorial Hospital, Danville, VA
12/71	-	M.S. from N.C. State University, Raleigh, NC
06/69	-	B.Sc. Agriculture, B.A. College of Agriculture, Anand, Gujarat State, India
06/65	-	S.S.C High School Certificate, Jeevan Bharti, Surat Gujarat State, India.

DISSERTATION TOPICS:

Penetration of a Series of Dialkoxy Analogs of Dimethoate Through the Isolated Gut of Insects and Mammals. (Under the direction of Dr. Frank Edwin Guthrie). A thesis submitted to the Graduate School at N.C. State University, Raleigh, NC; in partial fulfillment of the requirements for the degree of Master of Science.

Percutaneous Entry of Insecticides: A Comparative Study. (Under the direction of Dr. Frank Edwin Guthrie). A thesis submitted to the Graduate School at N.C. State University, Raleigh, NC; in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

ACADEMIC HONORS: Stood first in class during primary school years.

Secured first class in F.Y.B.Sc., S.Y.B.Sc., and final year B.Sc. in undergraduate school.

EXPERIENCE:

05/88 - Present: Industrial Toxicologist, Hoffmann-LaRoche, Inc., Nutley, NJ. Duties include health hazard assessment of intermediates and final products, monitoring acute toxicological studies, prepare toxicological section of MSDS's, make label recommendations, and develop risk assessment guidelines for the internal use.

12/86 - 05/88: Toxicologist, U.S. Environmental Protection Agency, Washington, DC. Primarily involved in health assessment of PMN and Existing chemicals, prepared guidelines for toxicological studies, reviewed studies submitted by the regulated industries and health criteria documents.

03/83 -12/86: Project Scientist, Northrop Services Inc., Research Triangle Park, NC. Developed a research project to study the percutaneous absorption and pharmacokinetics of toxic compounds into the mammalian physiological system, prepared proposals and final reports.

11/82 - 03/83: Consultant to Texas Tech University, Lubbock, Texas. Provided consulting services on dermal absorption of toxicants, to a Cooperative Agreement with the Environmental Protection Agency, RTP, NC.

08/74 - 06/82: Research Technician in Toxicology Program at N.C. State University, Raleigh, NC. Primarily involved in the research project related to percutaneous entry of toxicants, general supervision and maintenance of the laboratory, assist in teaching laboratory procedures to new students as well as students taking graduate level courses in entomology and toxicology.

12/73 - 08/74: Part-time Medical Technologist at the Memorial Hospital, Eden, North Carolina. Primarily involved in taking night calls and working in all phases of the clinical laboratory.

09/73 - 08/74: Chief Medical Technologist, the Memorial Hospital, Danville, Virginia. In charge of Autoanalyzer Sectio n. Duties included training and supervision of the personnel and maintenance of the equipment.

08/72 - 08/73: Student trainee in medical technology at the Memorial Hospital, Danville, Virginia. Primarily involved in the clinical training in all phases of the clinical laboratory such as chemistry, toxicology, microbiology, hematology, blood bank, urinalysis and outpatient laboratory.

05/72 - 08/72: Clinical Research Technologist at the Memorial Hospital, Danville, Virginia. Primarily involved in the method development for drugs screening.

08/70 - 05/72: Research Assistant in Toxicology Program at N.C. State University, Raleigh, North Carolina.

SCIENTIFIC SOCIETIES AFFILIATION: (Since)

Member of the Society of Toxicology (1979).

Member of the Mid-Atlantic Society of Toxicology (1988).

Member of Sigma XI (The Scientific Society), N.C. State University Chapter (1978).

Member of American Society of Clinical Pathologists (ASCP), (1974).

Member of American Industrial Hygiene Association, New Jersey Section, (1988).

PUBLICATIONS:

P.V. Shah, H.L. Fisher, N.J. Month, M.R. Sumler, and L.L. Hall. Percutaneous penetration of permethrin in Fischer 344 rats: Effects of age. (In preparation).

L.L. Hall, H.L. Fisher, M.R. Sumler and P.V. Shah. Percutaneous absorption and disposition of carbaryl in young and adult rats. (In preparation).

L.L. Hall, H.L. Fisher, M.R. Sumler and P.V. Shah. Age related percutaneous penetration of 2-sec-butyl 4,6-dinitrophenol (Dinoseb) in rats. Environ. Res. (submitted).

H.L. Fisher, P.V. Shah, M.R. Sumler, and L.L. Hall. Dermal penetration and pharmacokinetics of 14C captan in young and adult rats. (Submitted).

R. E. Grissom, Jr., and P. V. Shah. Cutaneous absorption of anticholinesterases. In. Clinical and Experimental Toxicology of Anticholinesterases, edited by B. Ballantyne and T. C. Marrs, Wright, Bristol (In press).

P.V. Shah, R.E. Grissom, Jr., and F.E. Guthrie. Environmental exposure to chemicals through dermal contact. In. Hazard Assessment of chemicals, Vol. 7, J. Saxena, ed., Hemisphere Publishing Corp., New York, 1990, pp 111-156.

L.L. Hall, and P.V. Shah. In vivo methods for measuring percutaneous absorption of xenobiotics: Indirect method. In. Methods for skin absorption. B.W. Kemppainen and W.G. Reifenrath, eds., CRC Press Inc., Boca Raton, FL. (In press).

P.V. Shah, H.L. Fisher, M.R. Sumler, and L.L. Hall. Dermal absorption and pharmacokinetics of pesticides in rats. In. Biological Monitoring for Pesticide Exposure: Measurement, estimation, and risk reduction, R.G. Wang, C.A. Frankline, R.C. Honeycutt, J. Reinert (eds), American Chemical Society, Washington, D.C., 1989, pp 169-187.

H.L. Fisher, P.V. Shah, M.R. Sumler, and L.L. Hall. In vivo and in vitro dermal penetration of 2,4,5-2'4'5' hexachlorobiphenyl in young and adult rats. Environ Res. 50: 120-139, 1989.

L.L. Hall, H.L. Fisher, M.R. Sumler, R.J. Monroe, N. Chernoff, and P.V. Shah. Dose response of skin absorption in young and adult rats. In: Performance of Protective Clothing, 2nd symposium, ASTM, STP-989, S.Z. Mansdorf, R. Sager, and A.P. Nielsen (eds.), American Society for Testing & Materials, Philadelphia, PA, 1988, pp 177-194.

P.V. Shah, H.L. Fisher, N.J. Month, M.R. Sumler, and L.L. Hall. Dermal penetration of carbofuran in young and adult Fischer 344 rats. J. Toxicol Environ. Health 22: 207-223, 1987.

P.V. Shah, H.L. Fisher, M.R. Sumler, R.J. Monroe, N. Chernoff, and L.L. Hall. A comparison of the penetration of fourteen pesticides through the skin of young and adult rats. J. Toxicol. Environ. Health 21:353-366, 1987.

P.V. Shah and F.E. Guthrie. Absorption of environmental contaminants. In: Reviews in Environmental Toxicology. E. Hodgson, ed., Elsevier, Amsterdam, New York, Oxford. Vol 2, 1-40, 1986.

P.V. Shah, D.J. Helling, B.P. Maliwal and F.E. Guthrie. Transport of conjugates of toxicant by blood proteins. Toxicol. Lett. 24:215-220, 1985.

P.V. Shah, M.R. Sumler, Y.M. Ioannou, H.L. Fisher, and L.L. Hall. Dermal absorption and disposition of 1,3-diphenylguanidine in rats. J. Toxicol. Environ. Health 15:623-633, 1985.

P.V. Shah and F.E. Guthrie. Dermal absorption of benzidine derivatives in rats. Bull. Environ. Conta. & Toxicol. 31:73-78, 1983.

P.V. Shah and F.E. Guthrie. Percutaneous penetration of three insecticides in rats: A comparison of two methods for in vivo determination. J. Invest. Dermatol. 80:291-293, 1983.

P.V. Shah, R.J. Monroe and F.E. Guthrie. Comparative penetration of insecticides in target and non-target species. Intern. J. of Drug and Chem. Toxicol. 6(2):155-179, 1983.

F.E. Guthrie, S.M., Ahdaya, P.V. Shah and B.P. Maliwal. Absorption and transport of insecticides in vertebrates. In. IUPAC, Pesticide Chemistry: Human Welfare and the Environment. S. Matsunaka, D.H. Hutson and S.D. Murphy, eds., Pergamon Press, Vol. 3, pp 451-456, 1983.

P.V. Shah, R.J. Monroe and F.E. Guthrie. Comparative rates of dermal penetration of insecticides in mice. Toxicol. Appl. Pharmacol. 59: 414-423, 1981.

P.V. Shah and F.E. Guthrie. Dermal absorption, distribution, and the fate of six pesticides in rabbits. In: Pesticide Management and Insecticide Resistance. D.L. Watson and A.W.A. Brown, eds., Academic Press, NY 1977, pp 547-554.

V.L. Patil, P.V. Shah and F.E. Guthrie. Absorption of insecticides in resistant and susceptible house flies. J. Econ. Entomol. 72 (3): 416-418, 1979.

S.M. Ahdaya, P.V. Shah and F.E. Guthrie. Thermoregulation in mice treated with parathion, carbaryl or DDT. Toxicol. Appl. Pharmacol. 35:575-580, 1976.

F.E. Guthrie, P.V. Shah and D.E. Moreland. Effects of pesticides on active transport of glucose through the isolated intestine of the mouse. J. Agr. Food Chem. 22 (4): 713-715, 1974.

P.V. Shah and F.E. Guthrie. Penetration of insecticides through isolated sections of the mouse digestive system: Effects of age and region of intestine. Toxicol. Appl. Pharmacol. 25: 621-624, 1973.

P.V. Shah, W.C. Dauterman and F.E. Guthrie. Penetration of a series of dialkoxy analogs of dimethoate through the isolated gut of insects and mammals. Pestic. Biochem. Physiol. 2 (3). 324-330, 1972.

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P.V. Shah, M.R. Sumler, H.L. Fisher, B.C. Edwards, S.P. Shrivastava, and L.L. Hall. Reproduceability of in vivo dermal absorption of pesticides in Fischer 344 rats. The Toxicologist 10(1):150, 1990.

B.D. Naumann, D.L. Conine, H.L. Hecker, E.V. Sargent, S.C. Gad, J.S. Mehring, L. Brooks, G.R. Koeing, P.V. Shah, J.P. Boehlert and T. White. Application of the PMA procedure for setting residue limits to methylene chloride. The Toxicologist 10(1):226, 1990.

S.P. Shrivastava, M.R. Sumler, H.L. Fisher, B.C. Edwards, P.V. Shah, and L.L. Hall. Reproduceability of in vitro dermal absorption of substituted phenols through rat skin. The Toxicologist 10(1):256, 1990.

L.L. Hall, H.L. Fisher, M.R. Sumler, S.P. Shrivastava, and P.V. Shah. Comparison of dermal penetration of pesticides determined by in vitro and in vivo methods in the rat. The Toxicologist 10(1):257, 1990.

L.L. Hall, H.L. Fisher, M.R. Sumler, S.P. Shrivastava, and P.V. Shah. Dermal absorption of folpet in young and adult rats. The Toxicologist 9(1):165, 1989.

S.P. Shrivastava, M.R. Sumler, H.L. Fisher, B.C. Edwards, M.F. Copeland, L.A. Oglesby, M.T. Eborn-McCoy, P.E. Beyer, R.J. Kavlock, P.V. Shah, and L.L. Hall. P-cyanophenol disposition in pregnant rats. The Toxicologist 9(1):236, 1989.

H.L. Fisher, P.V. Shah, M.R. Sumler, L.L. Hall. Dermal absorption and distribution of 14 C labelled captan in young and adult rats. Presented at Federation of American Society for Experimental Biology, May 1-5, 1988, Las Vegas, NE., Abstract #4462.

L.L. Hall, H.L. Fisher, M.R. Sumler, and P.V. Shah. Percutaneous penetration of nicotine in young and adult rats. The Toxicologist 8(1):123, 1988.

S.P. Shrivastava, H.L. Fisher, M.R. Sumler, P.V. Shah and L.L. Hall. Dermal absorption of disodium and monosodium methylarsenates (DSMA and MSMA) in young and adult rats. The Toxicologist 8(1),123, 1988.

P.V. Shah, H.L. Fisher, M.R. Sumler, M. Sanders, Y.M. Ioannou, and L.L. Hall. Dermal absorption and disposition of 1,2-Dihydro-2,2,4-trimethylquinoline in Fisher 344 Rats. The Toxicologist 7(1):244, 1987.

H.L. Fisher, L.L. Hall, P.V. Shah and M.R. Sumler. In vivo and in vitro Dermal penetration and pharmacokinetics of 2,4,5,2'4',5'-hexachlorobiphenyl in young and adult rats. The Toxicologist 7(1):243, 1987.

L.L. Hall, H.L. Fisher, M.R. Sumler and P.V. Shah. Age-related percutaneous penetration of dinoseb determined by in vivo and in vitro methods. The Toxicologist 7(1):243, 1987.

P.V. Shah, H.L. Fisher, M.R. Sumler, and L.L. Hall. Dermal penetration of carbofuran in young and adult Fischer 344 rats. The Toxicologist 6(1): 244, 1986.

H.L. Fisher, P.V. Shah, M.R. Sumler, and L.L. Hall. Chlordecone kinetics after dermal exposure. The Toxicologist 6(1):244, 1986.

L.L. Hall, H.L. Fisher, M.R. Sumler, and P.V. Shah. Age-related percutaneous penetration of carbaryl, determined by in vivo and in vitro methods. The Toxicologist 6(1):244, 1986.

P.V. Shah, H.L. Fisher, M.R. Sumler, and L.L. Hall. Skin penetration of fourteen pesticides in young and adult rats. The Toxicologist 5(1): 66, 1985.

L.L. Hall, H.L. Fisher, M.R. Sumler, and P.V. Shah. Dermal absorption and disposition of chlordecone in young and adult rats. The Toxiocologist 5(1):67, 1985.

P.V. Shah, Y.M. Ioannou, H.L. Fisher and L.L. Hall. Dermal absorption disposition of 1,3-diphenylguanidine in rats. The Toxicologist 4(1):145, 1984.

S.M. Ahdaya, P.V. Shah and F.E. Guthrie. Comparative penetration (in vivo) of insecticides through the skin and gastrointestinal tract of mice. Proceedings of the Seventeenth Annual Meeting of The Society of Toxicology, 1978.

P.V. Shah, S.M. Ahdaya and F.E. Guthrie. Absorption of pesticides from dermal and oral doses. Proceedings of North Carolina Academy of Science, Toxicology Section, 1978.

PAPERS PRESENTED AT SCIENTIFIC MEETINGS:

P.V. Shah, M.R. Sumler, H.L. Fisher, B.C. Edwards, S.P. Shrivastava, and L.L. Hall. Reproduceability of in vivo dermal absorption of pesticides in Fischer 344 rats. Accepted for the presentation at the 29 th Annual Meeting of The Society of Toxicology, in Miami Beach, Florida on February 12-16, 1990.

B.D. Naumann, D.L. Conine, H.L. Hecker, E.V. Sargent, S.C. Gad, J.S. Mehring, L. Brooks, G.R. Koeing, P.V. Shah, J.P. Boehlert and T. White. Application of the PMA procedure for setting residue limits to methylene chloride. Accepted for the presentation at the 29 th Annual Meeting of The Society of Toxicology, in Miami Beach, Florida on February 12-16, 1990.

S.P. Shrivastava, M.R. Sumler, H.L. Fisher, B.C. Edwards, P.V. Shah, and L.L. Hall. Reproduceability of in vitro dermal absorption of substituted phenols through rat skin. Accepted for the presentation at the 29 th Annual Meeting of The Society of Toxicology, in Miami Beach, Florida on February 12-16, 1990.

L.L. Hall, H.L. Fisher, M.R. Sumler, S.P. Shrivastava, and P.V. Shah. Comparison of dermal penetration of pesticides determined by in vitro and in vivo methods in the rat. Accepted for the presentation at the 29 th Annual Meeting of The Society of Toxicology, in Miami Beach, Florida on February 12-16, 1990.

L.L. Hall, H.L. Fisher, M.R. Sumler, S.P. Shrivastava, and P.V. Shah. Dermal absorption of folpet in young and adult rats. 28 th Annual Meeting of The Society of Toxicology, Atlanta, GA, February 27 - March 3, 1989.

S.P. Shrivastava, M.R. Sumler, H.L. Fisher, B.C. Edwards, M.F. Copeland, L.A. Oglesby, M.T. Eborn-McCoy, P.E. Beyer, R.J. Kavlock, P.V. Shah, and L.L. Hall. P-cyanophenol disposition in pregnant rats. 28 th Annual Meeting of The Society of Toxicology, Atlanta, GA, February 27 - March 3, 1989.

H.L. Fisher, P.V. Shah, M.R. Sumler, L.L. Hall. Dermal absorption and distribution of 14 C labelled captan in young and adult rats. Presented at Federation of American Society for Experimental Biology, May 1-5, 1988, Las Vegas, NE., Abstract #4462.

L.L. Hall, H.L. Fisher, M.R. Sumler, and P.V. Shah. Percutaneous penetration of nicotine in young and adult rats. 27th Annual Meeting of The Society of Toxicology, Dallas, TX. February 15-19, 1988.

S.P. Shrivastava, H.L. Fisher, M.R. Sumler, P.V. Shah and L.L. Hall. Dermal absorption of disodium and monosodium methylarsenates (DSMA and MSMA) in young and adult rats. 27th Annual Meeting of The Society of Toxicology, Dallas, TX. February 15-19, 1988.

P.V. Shah, H.L. Fisher, M.R. Sumler and L.L. Hall. Dermal absorption and pharmacokinetics of pesticides in rats. 194th ACS National Meeting, New Orleans, LA. August 30 - September 4, 1987.

P.V. Shah, H.L. Fisher, M.R. Sumler, M. Sanders, Y.M. Ioannou, and L.L. Hall. Dermal absorption and disposition of 1,2,-dihydro-2,2,4-trimethylquinoline in Fischer 344 rats. 26th Annual Meeting of the Society of Toxicology, Washington, DC. February 24-27, 1987.

H.L. Fisher. L.L. Hall, P.V. Shah, and M.R. Sumler. In vivo and in vitro dermal penetration and pharmacokinetics of 2,4,5-2',4',5'-hexachlorobiphenyl in young and adult rats. 26th Annual Meeting of the Society of Toxicology, Washington, DC. February 24-27, 1987.

L.L. Hall, H.L. Fisher, M.R. Sumler and P.V. Shah. Age related percutaneous penetration of dinoseb determined by in vivo and in vitro methods. 26th Annual Meeting of the Society of Toxicology, Washington, DC, February 24-27, 1987.

P.V. Shah, H.L. Fisher, M.R. Sumler, and L.L. Hall. Dermal penetration of carbofuran in young and adult Fischer 344 rats. 25th Anniversary Meeting of the Society of Toxicology, New Orleans, LA, March 3-7, 1986.

H.L. Fisher, P.V. Shah, M.R. Sumler, and L.L. Hall. Chlordecone kinetics after dermal exposure. 25th Anniversary Meeting of the Society of Toxicology, New Orleans, LA, March 3-7, 1986.

L.L. Hall, H.L. Fisher, M.R. Sumler, and P.V. Shah. Age-related percutaneous penetration of carbaryl, determined by in vivo and in vitro methods. 25th Anniversary Meeting of the Society of Toxicology, New Orleans, LA, March 3-7, 1986.

P.V. Shah, H.L. Fisher, M.R. Sumler, and L.L. Hall. Skin penetration of fourteen pesticides in young and adult rats. 24th Annual Meeting of the Society of Toxicology, San Diego, CA, March 18-22, 1985.

L.L. Hall, H.L. Fisher, M.R. Sumler, and P.V. Shah. Dermal absorption and disposition of chlordecone in young and adult rats. 24th Annual Meeting of the Society of Toxicology, San Diego, CA, March 18-22, 1985.

P.V. Shah, Y.M. Ioannou, H.L. Fisher and L.L. Hall. Dermal absorption and disposition of 1,3-diphenylguanidine in rats. 23rd Annual Meeting of the Society of Toxicology, Atlanta, GA, March 12-16, 1984.

F.E. Guthrie, S.M. Ahdaya, P.V. Shah and B.P. Maliwal. Absorption and transport of insecticides in vertebrates. The Fifth International Congress of Pesticide Chemistry (IUPAC) August 29, 1982, Kyoto, Japan.

P.V. Shah, S.M. Ahdaya and F.E. Guthrie. Comparative absorption of insecticides via dermal and oral routes in mice. Second Annual Meeting of North Carolina Society of Toxicology, Research Triangle Park, NC, February 1982.

S.M. Ahdaya, P.V. Shah and F.E. Guthrie. Comparative penetration (in vivo) of insecticides through the skin and gastrointestinal tract of mice. 17th Annual Meeting of the Society of Toxicology, San Francisco, CA, March 12, 1978.

P.V. Shah, S.M. Ahdaya, and F.E. Guthrie. Absorption of pesticides from oral doses. North Carolina Academy of Science, Toxicology Section, Winston-Salem, NC, 1978.

P.V. Shah and F.E. Guthrie. Dermal absorption of insecticides in rabbits. International Congress of Entomolog, Washington, D.C., August, 1976.

REFERENCES:

Dr. E. Hodgson
Toxicology Program
P.O. Box 5215
N. C. State University
Raleigh, North Carolina 27650
919-737-2274

Dr. Larry L. Hall
U.S. Environmental Protection Agency
Developmental Biology Division
Research Triangle Park, North Carolina 27711
919-541-2774

Dr. Neil Chernoff
U.S. Environmental Protection Agency
Developmental Biology Division
Research Triangle Park, North Carolina 27711
919-541-2651

Roche Hoffmann-La Roche
A Member of the Roche Group

Hoffmann-La Roche Inc
340 Kingsland Street
Nutley New Jersey 07110 1199

Jack S. Kace. Eng. Sc.D.
Vice President and Director
Environmental & Safety Affairs
Tel 201-235-3774

September 26, 1995

GENERAL COMPLIANCE STATEMENT

Hoffmann-La Roche Inc does not expect that approval of NDA 20-628 for INVIRASE (saquinavir) Capsules will have any effect upon current emission requirements at each of the production sites

Signed Jack Kace
Jack Kace

Date 9/26/95

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

IND #:20-628

REVIEWER	: N. Battula
CORRESPONDENCE DATE	: 08-31-95
CDER RECEIPT DATE	: 08-31-95
REVIEW ASSIGN DATE	: 09-01-95
REVIEW COMPLETE DATE	: 12-06-95

SPONSOR: Hoffmann-La Roche Inc.
340 Kingsland Street
Nutley, New Jersey 07110

SUBMISSION REVIEWED: Original

DRUG CATEGORY: Anti-HIV (protease inhibitor)

INDICATION: Monotherapy and combination treatment (with ddC and/or ZDV) for patients with advanced HIV infection

DOSAGE FORM: Oral. Capsules. 200 mg.

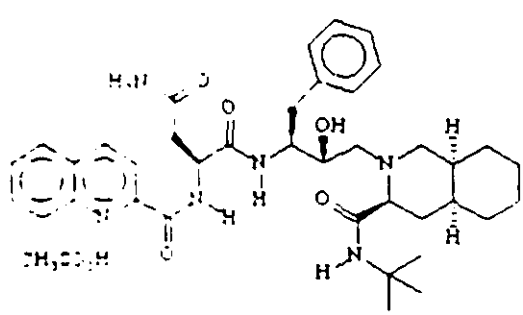
PRODUCT NAMES:

a. PROPRIETARY: Invirase [TM]

b. NONPROPRIETARY: Saquinavir

c. CHEMICAL: *cis*-N-*tert*-Butyl-decahydro-2[2(**R**)-hydroxy-4-phenyl-3(**S**)-{[N-(2-quinolylcarbonyl)-**L**-asparginyl]amino]butyl] -(4a**S**,8a**S**)-isoquinoline-3(**S**)-carboxamide methanesulfonate

STRUCTURAL FORMULA:



SUPPORTING DOCUMENTS: IND

BACKGROUND: This original New Drug Application on Invirase™ (previously used name, Saquinavir), submitted by Hoffmann-La Roche is proposed for the treatment of HIV infections in selected patients. Invirase is a rationally designed synthetic inhibitor of virus encoded enzyme, the HIV protease. The applicant is seeking the endorsement of the FDA for invirase under the Accelerated Approval of New Drugs for Serious or Life-Threatening Illnesses. The proposed indication is for monotherapy of advanced HIV disease in patients intolerant to currently available therapies, and for combination therapy with Hivid® and/or Retrovir® for the treatment of advanced HIV disease in selected patients with CD_4+ T cell count of $\leq 300/mm^3$. The requested indication is based on the results of immunologic and virologic surrogate markers.

Human immunodeficiency virus, the etiological agent of the acquired immunodeficiency syndrome is an RNA virus that replicates through a DNA intermediate. The DNA copy of the viral RNA (proviral DNA) integrates into the cellular DNA (forming the provirus), thus establishing the viral infection. Transcription of the proviral DNA and translation of the viral transcripts by the combined action of the host cell and virus coded functions results in the production of the progeny HIV. Thus, the HIV replication cycle can be divided into two phases. The pre-integration phase in which viral DNA synthesis is carried out by the HIV reverse transcriptase, an enzyme which comes prepackaged in the virion, and the post-integration phase in which the progeny infectious virus is produced by the combined action of both the host and virus coded functions. The virus encoded functions include the HIV protease, the activity of which is indispensable for the production of the infectious virus. This critical role of the protease essential for the production of infectious HIV resulted in the recognition of the protease enzyme as a highly attractive therapeutic target for therapeutic intervention in the treatment of HIV infections..

All of the currently approved anti-HIV chemotherapeutic agents act by inhibiting reverse transcriptase-directed viral DNA synthesis that is essential for establishing viral infection. As such these drugs can only affect one part of the virus life cycle mediated by viral RT i.e., inhibit virus spread by blocking new rounds of infections only (inhibit acute infection). HIV RT inhibitors, however, should have no effect on already established infections (chronic infection) from which HIV replication can continue. Therefore nucleoside analogues, at best can reduce the virus load incompletely by blocking acute infection but have no effect on the virus production from the large reservoir of already infected cells in HIV positive subjects. Therefore, there is a great need for additional drugs or drug-combinations that offer greater decrease in virus load either by

monotherapy or by combination therapy. In this context targeting of HIV protease may offer greater advantage since inhibition of this enzyme blocks virus production from both acutely and chronically infected cells.

HIV, in the course of its replication cycle, produces polycistronic mRNAs whose long *gag* and *pol* polyprotein products must be specifically cleaved to generate the individual functional proteins found in the infectious HIV. This specific proteolytic cleavage is brought about by the HIV-encoded proteolytic enzyme, the HIV protease. The protease is a 99 amino acid peptide that dimerizes to form the active enzyme. The active dimeric protease processes the long *gag* polyprotein precursor, $p55^{gag}$, to generate the mature *gag* proteins; p27, p24, p9 and p7, which constitute the viral core structure. The protease also processes the *gag-pol* fusion polyprotein $p160^{gag\text{-}pol}$, to produce the viral enzymes; the viral protease itself, reverse transcriptase, ribonuclease H and integrase, all of which are essential to HIV replication. The processing of these polyprotein precursors require at least ten peptidolytic cleavages and these cut sites in *gag* and *pol* are diagrammatically presented in Figure 1.

HIV protease is classified as an aspartyl enzyme by virtue of it containing the signature catalytic site sequence Asp-Thr-Gly residues which are directly involved in the catalytic cleavage of the *gag* and *pol* polyprotein precursors. The indispensable nature of this enzyme for the production of infectious HIV was demonstrated by several criteria including the introduction of a point mutation in the active site which renders the enzyme inactive and by blocking the enzyme activity with the use of inhibitors. Both of these methods blocked processing of the polyproteins and as a consequence morphologically aberrant non-infectious virus particles were produced. An abundance of data on the biological and biochemical understanding of the enzyme coupled with the simultaneous availability of the 3-dimensional structure from X-ray crystallography studies facilitated a detailed understanding of the catalytic mechanism of the enzyme action. The detailed understanding of the structure-activity relationships of the HIV protease greatly assisted the discovery of many rationally designed inhibitors of the enzyme. Currently, a number of such rationally-designed synthetic inhibitors of the HIV protease are investigational drugs. Invirase is the first NDA of what appears to be one of a series of HIV protease inhibitor drug applications expected to be submitted to the FDA

Saquinavir, the subject of this NDA, is a non-hydrolyzable peptide mimetic designed to serve as surrogate substrate for HIV protease. It is contrived to imitate the transition-state configuration adopted by the natural substrate amino acids at the scissile peptide bond that is subject to cleavage by the viral enzyme. The protease is an unusual enzyme in that it does not carry out a single reaction at a specific rate but has loose specificity and evolved to recognize many different sequences and cuts at as many as 10 to 12 different sites in a specific and orderly manner. To derive maximal inhibitory benefit, the sponsor optimized invirase to mimic the configuration between the aromatic amino acids and proline, namely Phe Pro or Tyr-Pro, since three of the cleavage sites on the polyprotein substrates have this structure (see Fig 1). Thus, the mechanism based peptidomimetic invirase is designed to competitively inhibit the HIV protease activity with the expectation of blocking the formation of functional viral proteins that are essential for the infectivity of HIV.

In the microbiology portion of the review a general summary of the preclinical microbiology studies is provided. The studies include: (1) anti-protease activity and specificity of invirase analyzed by using purified HIV-protease and representative mammalian proteases (2) antiviral activity in acute and chronic infections (3) antiviral activity in combination with other anti-HIV agents (4) drug-sensitivity profiles (phenotypic and genotypic resistance) in monotherapy and in combination therapy with other anti-HIV agents, and (5) mechanism of action.

SUMMARY

Anti-protease activity of saquinavir: Recombinant proteases of HIV-1 and HIV-2 expressed in *Escherichia coli* were purified. The activity of the enzymes and their inhibition by saquinavir was determined using a synthetic hepta-peptide substrate, succinyl.Val.Ser.Asn.Phe.Pro.Ile.iso-butylamide as well as by using recombinant natural substrates: the *gag* polyproteins and the *pol* polyproteins that have been expressed in a baculovirus expression system.

In the case of the synthetic substrate, the proteolytic cleavage products of the Phe.Pro bond produces an N-terminal proline which when reacted with isatin forms a chromogenic product that was quantified spectrophotometrically. The concentration of saquinavir required to inhibit the activity of the protease by 50% and 90% was determined. In this test system the 50% inhibitory concentrations (I_{50}) for HIV-1 and HIV-2 proteases were 0.4 nM and 0.8 nM, respectively.

The K_i values for HIV-1 and HIV-2 proteases were 0.21 nM and < 0.1 nM, respectively.

In the case of natural substrates, the proteolytic cleavage of the polyproteins by purified HIV-1 and HIV-2 proteases was diagnosed by the size shift of p24 (cleaved from p55 to produce p24) on immunoblots detected by antibodies against p24 antigen. The analysis suggests that saquinavir inhibited the cleavage of the polyprotein substrates in a dose dependent manner. These studies with peptides and proteins were performed under kinetically optimal (K_m and K_{cat}) conditions of low pH and high salt. Invirase inhibited the proteolytic cleavage in a dose related manner and at 1 μM it completely blocked the processing of the HIV-gag polyprotein of both HIV-1 and HIV-2 proteases. The polyprotein cleavage assay is a qualitative assay.

The total length of HIV polyprotein precursors subject to processing by the viral protease is approximately 1500 amino acids. The protease enzyme recognizes about 10 to 12 different sequences as processing sites on the polyproteins and cleaves the sites at different rates (the rates of cleavages for different sites vary up to 400 fold). In vitro cleavage studies with synthetic peptide and polyprotein substrates were performed under kinetically optimal conditions of low pH and high salt which were not representative of the physiological conditions in which the cleavages and inhibitions occur in vivo. Furthermore, in the peptidolytic assay using the synthetic substrate only the minimal length of 7 amino acid residues required for cleavage by the proteases was used and this situation does not take into account the protein folding and domain structures of polyproteins. However, the simplicity of the assay makes it useful for routine evaluation of potential protease inhibitors. These enzyme inhibition studies do indicate the proof of principle and the relative inhibitory strengths of the test compounds, an important step in the drug development process.

Specificity of saquinavir: Humans contain numerous proteases and they have been classified into aspartyl, serine, cysteine and metallo proteases with each class containing multiple enzymes. HIV protease is an aspartyl protease and shares structural and mechanistic properties with human aspartyl proteases. In order for saquinavir to be useful in the clinic it should not only be active against the viral protease but should ideally also show little or no effect on the cellular proteases at concentrations which will be attained within patients at the treated doses. An expectation of a molecularly targeted and rationally designed therapeutic such as saquinavir is that such specific agents do not elicit side effects such as cellular toxicity, at least not mechanistic toxicity. The

sponsor tested the inhibitory effect of saquinavir on representative members of the 4 classes of cellular proteases and the data are presented in Table 1

Table 1. Selectivity inhibition of HIV protease by Saquinavir

PROTEASE	CLASS	ACTIVITY (nM)
HIV-1 Protease	Aspartic	IC_{50} = 0.4 Ki = 0.12
HIV-2 Proteinase	Aspartic	IC_{50} < 0.8, Ki < 0.1
Human Renin	Aspartic	IC_{50} > 10,000
Human Pepsin	Aspartic	IC_{50} > 10,000
Human Gastricsin	Aspartic	IC_{50} > 10,000
Human Cathepsin D	Aspartic	IC_{50} > 10,000
Human Cathepsin E	Aspartic	IC_{50} > 10,000
Human Leucocyte Elastase	Serine	IC_{50} > 10,000
Bovine Chymotrypsin	Serine	IC_{50} > 10,000
Bovine Cathepsin B	Cysteine	IC_{50} > 10,000
Human Synovial Fibroblast Collagenase	Metallo	IC_{50} > 10,000

The data in Table 1 show that saquinavir at concentrations greater than 10 μM was less than 50% inhibitory to human aspartic proteases as well as serine, cysteine, and metallo protease tested. The high inhibitory concentration toward cellular proteases which is greater than 10^5 fold to that of the HIV protease, indicates that saquinavir is a specific inhibitor of the HIV-1 protease. By extrapolation of this limited data the sponsor suggested that saquinavir may have low potential for adverse effects. The enzymes tested are too few to arrive at such conclusion. However, it is not feasible to test the inhibitory activity of saquinavir against all known human proteases.

Antiviral Activity of Saquinavir *Test systems and assays:* In order to lend support and perspective to the antiviral activity profile of saquinavir the sponsor examined the antiviral activity of saquinavir in a variety of host cell virus strain combinations. The host cell lines tested include

different lymphocytic cell lines (JM, CEM, 174XCEM, sup-T1, C8166, AA2, VB, MT-4, MT-2,), monocyte-macrophages and peripheral blood lymphocytes. The viruses used to infect the cell lines include laboratory-passaged lymphotropic HIV strains selected from different geographic locations; HIV-1_{GB8}, HIV-1_{RF}, HIV-1_{RF}, HIV-1_{SF}, HIV-1_{SF}, HIV-1_{HWS2}, HIV-1_{LAV}, HIV-1_{IIIB} HIV-1_{111-B}, clinical isolates and drug resistant isolates

End points: Standard assay methods conventionally used in the determination of antiviral HIV activities by drugs were employed in these studies and they were essentially of two kinds: (1) assays based on the yield of viral components in the culture medium: In this system three viral components were usually measured, virion associated RT activity (radioisotope incorporation measurements), viral p24 (immune assay) and viral RNA (quantitative PCR), and (2) assays based on cell damage due to infection: generally two effects on infected cells were measured, syncytial cell formation in which adjacent cells fuse due to the action of virion gp120 to form giant cells and cytopathic effect (CPE) or cell death. CPE is generally determined by counting the remaining viable cells electronically or by measuring selective dye uptake such as trypan blue or by measuring the metabolism of the cells using the MTT dye assay. The advantage of the MTT assay is that it allows a parallel assessment of the antiviral and cytotoxic effects of test compounds in virus infected cells and in parallel mock-infected cells, respectively.

HIV RNA was quantified by the method of reverse transcription coupled to DNA polymerase chain reaction (RT-PCR), developed by the Roche Molecular Systems. Based on its design, the assay appears to be specific, sensitive and reproducible with a 4-5 log unit dynamic range for RNA quantification. The lower detection limit of viral RNA by this assay is 200 copies/ml, which corresponds to 100 HIV virions. The major deficiency of the assay is that it measures the physical amount of a 142 base viral RNA out of approximately 9200 base long HIV RNA and does not give a clue as to its functionality or dysfunctionality. Furthermore, comparison of similar RNA copy number changes as equivalent in response to treatment with different drugs classes that exert antiviral activity through different modes of action could be misleading. For example, a similar change in viral RNA in response to RT inhibitor drug therapy and protease inhibitor drug therapy although biochemically similar, they functionally could be vastly different. The protease inhibitors should have no effect on the production of the virion itself or the packaging of the viral RNA but the virus produced is non-infectious. RT inhibitors on the other hand inhibit virus infection and consequently the virus production. Thus a similar viral RNA copy number in the former case

represents an over estimate of the infectious virions but in the latter case it represents a true estimate. In spite of the draw backs this RT-PCR is currently the most widely used and accepted assay for quantifying HIV RNA.

End-point measures: The concentrations of the test compound required to reduce each of the virus-induced effects (such as p24, syncytia etc.) by 50% and 90% are referred to as IC_{50} and IC_{90} respectively. These values were determined graphically from plots of inhibition against concentration of the test compound. Similarly, the cytotoxicity of the test compounds were determined by plotting cytotoxicity against concentration of the test drug. The values were expressed as ID_{50} and ID_{90} (i.e., concentrations of drug producing 50% and 90% cytotoxicity in cell culture, respectively). Therapeutic Index (TI) is a calculated value which represents the ratio of cytotoxicity, ID_{50} , to antiviral activity, IC_{50}.

The sponsor evaluated the in vitro antiviral activity of saquinavir in acute infection assay systems in which the cells were infected and treated for a relatively short period of time (about 3 days) and in chronic infection assay systems in which viral infection was already well established in the cell culture before treatment, allowing a different appreciation of saquinavir's ability to intervene against ongoing infection. Results of these two perspectives of viral infection are presented separately.

Antiviral activity in acute infection: The antiviral activity of saquinavir was dependent on multiple factors such as the host cell, virus strain, the multiplicity of infection, and the endpoint used. In addition, differences between HIV strains at the level of gene sequences coding for the viral protease, the target of saquinavir, could lead to strain differences. To avoid these biases and lend support and perspective to the antiviral activities of saquinavir the sponsor examined the antiviral activities in different host cell/virus strain combinations to approximate the balance of cells and viruses in vivo. The antiviral activities in acute infections using laboratory strains of HIV are summarized in tables 2 to 5. Each of these tables is arranged to reflect a single assay end point measured in different host cell/virus combinations. Accordingly, tables 2, 3, 4, and 5 show antiviral activities determined by syncytial assay, MTT dye assay, p24 assay and RT assay, respectively.

Table 2 shows the results of syncytial reduction assay in CEM and JM cells infected with HIV-1 strains GB8 and MN. In this test system the multiplicity of infection of 0.005 syncytium forming units/cell was used and the syncytia were scored by light microscopy after 3 days.. Half-log dilutions of saquinavir in the range of 0.3-100 nM was used and percent inhibition of syncytium formation was calculated by reference to control untreated cultures.

Table 2. Antiviral activity against laboratory HIV isolates

Virus strain	Cell host	Antiviral activity (nM)	
		saquinavir	Standard
$HIV\text{-}1_{GB8}$	JM (1)	$IC_{50}=3.1$ $IC_{90}=14$	ZDV inactive at 1000
$HIV\text{-}1_{GB8}$	JM (2)	$IC_{50}=2.3$ $IC_{90}=18$	ZDV inactive at 1000
$HIV\text{-}1_{GB8}$	CEM	$IC_{50}=6.5$ $IC_{90}=25$	ZDV $IC_{50}=9.3$ $IC_{90}=110$ R82150 $IC_{50}=31$ $IC_{90}=210$
$HIV\text{-}1_{GB8}$	CEM	$IC_{90}=28$	ZDV $IC_{90}=62$ ddC $IC_{90}=240$
$HIV\text{-}1_{GB8}$	Sup-T1	$IC_{50}=2.0$ $IC_{90}=6.0$	n.d.*
$HIV\text{-}1_{MN}$	JM	$IC_{50}=4.0$ $IC_{90}=22$	n.d.

*n.d. not determined in comparative studies with other antiviral agents
(1) and (2). data from separate experiments

The results in Table 2 show that the IC_{50} values were in the range of 2 to 6.5 nM and the IC_{90} values were in the range of 6 to 28 nM The antiviral activity of saquinavir was greater than that

of the nucleoside analogues AZT and ddC and the nonnucleoside analogue R82150. AZT, as expected, was inactive in JM lymphoid cells which are thought to lack phosphorylating enzyme for this precursor.

The antiviral activity o. ir as determined by the MTT dye reduction assay is presented in Table 3. In parallel experiments nucleoside analogues ZDV, ddC and ddI were used for a comparative evaluation of the antiviral activity of saquinavir.

Table 3. Antiviral activity against laboratory HIV isolates

Virus strain	Cell host	Antiviral activity (nM)	
		saquinavir	Standard
HIV-1_{GB8}	Sup-T1	IC_{50}=7.0 IC_{90}=23	ZDV IC_{50}=18 IC_{90}=160
HIV-1_{NIT}	MT-4	IC_{50}=28	ZDV IC_{50}=13 ddC IC_{50}=310
HIV-1_{RF}	MT-4	IC_{50}=4.7 IC_{90}=7.8	ddI IC_{50}=5300 IC_{90}=10000
HIV-1_{RF}	MT-4	IC_{50}=6.0	ZDV IC_{50}=30
HIV-1_{IIIB}	MT-4	IC_{50}=14	ZDV IC_{50}=0.5 ddI IC_{50}=4800

The results in Table 3 show that the IC_{50} and IC_{90} for saquinavir were in the range of 4.7 to 28 nM and 7.8 to 25 nM, respectively. Comparative evaluation of the data suggest that saquinavir is a better antiviral agent than the nucleoside analogues.

The antiviral activity of saquinavir as measured by inhibition of p24 antigen production in culture supernatant fluids is presented in Table 4. Results of antiviral activity of nucleoside analogues determined in parallel experiments are also presented.

Table 4. Antiviral activity against laboratory HIV isolates

Virus strain	Cell host	Antiviral activity (nM)	
		Saquinavir	Standard
$HIV\text{-}1_{GB8}$	CEM	IC_{90} = 3.3 IC_{99} = 15	ZDV IC_{90} = 5.3 IC_{99} = 37
$HIV\text{-}1_{GB8}$	CEM	IC_{95} = 15	ZDV IC_{90} = 25 ddC IC_{90} = 120
$HIV\text{-}1_{GB8}$	CEM	IC_{90} = 3.6	ZDV IC_{50} = 5.3
$HIV\text{-}1_{GB8}$	174XCEM	IC_{90} = 1.9 IC_{99} = 7.0	n.d.
$HIV\text{-}1_{GB8}$	Sup-T1	IC_{90} = 1.7 IC_{99} = 3.9	n.d.
$HIV\text{-}1_{GB8}$	C8166	IC_{90} = 3.6 IC_{99} = 8.2	n.d.
$HIV\text{-}1_{GB8}$	AA2	IC_{90} = 1.1	n.d.
$HIV\text{-}1_{GB8}$	VB	IC_{90} = 2.0 IC_{99} = 6.5	n.d.
$HIV\text{-}1_{RF}$	AA2	IC_{90} = 0.9	Z DV IC_{90} = 2.2
$HIV\text{-}1_{NIT}$	CEM	IC_{90} = 1.5 IC_{99} = 3.1	ddI IC_{50} = 4200 IC_{90} = 1500
$HIV\text{-}1_{HXB2}$	MT-2	IC_{90} = 1.7 IC_{99} = 8.9	n.d.
$HIV\text{-}1_{Ba\text{-}L}$	MM	IC_{90} = 20 IC_{99} = 200	ZDV IC_{50} = 10 IC_{90} = 60
$HIV\text{-}1_{RF}$	C8166	IC_{90} = 2	ZDV IC_{90} = 10 ddC IC_{90} = 200
$HIV\text{-}2_{ROD}$	C8166	IC_{90} = 4	n.d.
$HIV\text{-}1_{IIIB}$	C8166	IC_{90} = 0.3	ZDV IC_{90} = 100
$HIV\text{-}1_{Ba\text{-}L}$	MM	IC_{90} = 1.4 IC_{99} = 4.6	ZDV IC_{50} = 6 IC_{99} = 100 ddI IC_{50} = 50 IC_{99} = 1000 ddC IC_{50} = 1 IC_{90} = 10

The results in Table 4 show that the IC_{50} and IC_{90} for saquinavir are in the range of 1.1 to 20 nM and 0.3 to 200 nM, respectively. The antiviral activity of saquinavir as measured by inhibition of p24 production is similar to that of ZDV but is more active than ddC and ddI.

Table 5 shows the antiviral activity of saquinavir as measured by RT from cell culture supernatants. In PBMC the antiviral activity of saquinavir and ZDV was similar with IC_{90} value of 7 and 4 nM, respectively. The IC_{90} of ZDV in the case of monocyte/macrophage cell line OM-10.1 , however, was much higher (> 100) nM) than for saquinavir. The low inhibitory effect of ZDV is attributed to low levels or lack of phosphorylating enzymes required for the antiviral effect of ZDV.

Table 5.Antiviral activity determined by measuring viral RT

Virus strain	Host cell	Antiviral Activity (nM)	
		Saquinavir	Standard
HIV-1	PBMC	IC_{90} = 7	ZDV IC_{90} = 4
HIV-1	OM-10.1	IC_{90} = 9	ZDV IC_{90} = >100

In one study the sponsor assessed the antiviral activity of saquinavir by determining the infectious virus yield in lymphocytic cells, C8166, infected with HIV-1 $_{IIIB}$ The IC_{50} and IC_{90} values by this assay were 1.7 and 6.4 nM, respectively Comparative antiviral activities using other antiviral agents were not reported in this study

The antiviral effect of saquinavir in primary monocyte-macrophage cells was compared with other antiviral compounds and the results are presented in Table 6. Monocytes isolated from freshly prepared PBMC by adherence on gelatin-coated cell culture plates were infected with macrophage tropic HIV-1 strain Ba-L and the viral p24 antigen production was determined 9 days after

infection. The antiviral activity data presented is an average of three different experiments. For each drug tested for antiviral activity cell viability was determined in parallel by trypan blue exclusion.

Table 6. Comparative activities against HIV-I_{MN} in monocyte-macrophages

Drug	nM	
	IC_{50}	IC_{90}
Saquinavir	20	200
ZDV	2	60
ddI	120	890
ddC	0.3	5
Nevirapine	70	1610

The results in Table 6 show that saquinavir inhibits HIV replication in monocyte-macrophages but is less active than ZDV and ddC and some what better than ddI. The nonnucleoside analogue RT inhibitor, neverapine, was less inhibitory than saquinavir.

Antiviral activity of saquinavir on clinical isolates: The sponsor determined the antiviral activity of clinical isolates from patients and also paired isolates pre and post exposure to ZDV. The antiviral activity of saquinavir as determined by the inhibition of viral p24 antigen production in the culture supernatant of cells infected with clinical HIV isolates for infection is presented in Table 7.

Table 7. Activity of saquinavir against HIV-1 clinical isolates

Virus	Cell host	Antiviral activity	
		Saquinavir (nM)	Standard (nM)
14a - Pre	PBMC	$IC_{90} = 10$ $IC_{95} = 20$	ZDV $IC_{90} = 10$ $IC_{95} = 50$ ddI $IC_{90} = 500$ $IC_{95} = 2000$
14a - Pre	PBMC	$IC_{95} = 24$	ZDV $IC_{90} = 100$ ddI $IC_{90} = 4000$
14a - 4/87	PBMC	$IC_{50} = 3.5\text{-}9.1$	ZDV $IC_{50} = 0.3$ ddI $IC_{50} = 670$
14a - 6/89	PBMC	$IC_{90} = 6.2$	ZDV $IC_{90} = 370$ ddI $IC_{90} = 920$
"Isolate B"	PBMC	$IC_{90} = 10$	ZDV $IC_{90} = 100$
"Isolate D"	PBMC	$IC_{90} = 3.0$	ZDV $IC_{90} = 100$

14a-pre, 14a-4/87 and 14a-6/89 are matched isolates from the same patient at pretreatment, 26 week of treatment and long term (> 26 weeks) treatment with AZT. Isolate B and isolate D are HIV-1 isolates from AZT-naive and AZT-experienced (treated for 14 months) patients, respectively.

The results in Table 7 show that saquinavir was active against clinical isolates and the concentration of the drug required to inhibit the activity was similar to the laboratory isolates of HIV-1. The AZT resistant isolates are equally sensitive to saquinavir as were the AZT-sensitive isolates, indicating no cross-resistance between the RT inhibitors and the protease inhibitor, saquinavir.

Antiviral activity in chronically infected cells: Saquinavir, unlike nucleoside analogues, is a direct acting drug and therefore should be effective inside all HIV producing cells regardless of their metabolic status. The sponsor tested the ability of saquinavir for antiviral activity in chronically infected cells which constitutively produce HIV from the proviral DNA. JM cells chronically infected with HIV-1 strain GB8 were mixed with uninfected cells (ratio 1:100) and assayed for

inhibition of syncytium formation by saquinavir. The mean IC_{50} and IC_{90} were 2.7 nM and 16 nM, respectively. In parallel experiments AZT was inactive and this was attributed to a lack of thymidine kinase activity in JM cells.

HIV protease is involved in the processing of viral polyproteins and maturation of infectious virus. The sponsor investigated the inhibitory ability of saquinavir in polyprotein processing and maturation of infectious virus. CEM cells chronically infected with HIV-1 strain IIIB were assayed for the cleavage product p24 from its polyprotein, p55, by immunoblotting and identifying the p24 product by its antibodies and its size shift on the blot. Examination of the immunoblot showed inhibition of p24 production at saquinavir concentrations as low as 0.01 nM and it was progressive attaining barley detectable levels at a saquinavir concentration of 0.3 nM and undetectable at concentrations of 1 nM and above. In spite of the inhibition of the polyprotein processing by saquinavir, budding of virions from the cell membranes is expected to continue. Electron microscopy examination of the virions budding from saquinavir treated cultures showed 'donut like' immature virions lacking characteristic dark inner core component found in mature infectious virus. This observation supports that saquinavir prevents the physical maturation of HIV virions and is effective in chronic infections. Inhibition of HIV maturation by saquinavir was also demonstrated by electron microscopy in U1 monocytic cell cultures which extended the formation of immature virions in host cells of a different lineage.

The infectivity of the HIV virions produced in the presence of saquinavir was determined by titration on CEM cells. At a saquinavir concentration of 20 and 100 nM the infection titer was reduced by 100 and 200 fold compared to the untreated control titer. These results confirm that the immature virions formed in the presence of saquinavir are either non-infectious or have very low infectivity.

The combined results from biochemical experiments showing inhibition of polyprotein processing, morphological evidence of the formation of immature virions by electron microscopy and the biological evidence of loss of infectivity in the presence saquinavir support the conclusion that saquinavir is an inhibitor of HIV protease activity. The inhibitor is effective in chronically infected cells and, thus, is distinguishable from nucleoside analogues.

Antiviral activity of saquinavir in combination with other anti-HIV agents: Treatment of HIV infections with single antiviral agents have been associated with development of drug related toxicities, relatively small decreases in virus load, emergence of resistance and perhaps clinical failure. The rationale for combination therapy is to provide greater viral suppression, decrease toxicities by decreasing dose and limit the emergence of drug resistance. This goal may be achieved by combination of agents that employ distinct modes of action and having non-overlapping resistance profiles. Therefore, combination therapy with RT inhibitors and protease inhibitors which exert antiviral activity through distinct modes of action on two different viral targets and at two different stages of the virus replication cycle offer a sound rationale for prolonged viral suppression and decrease in the emergence of resistance.

The sponsor investigated the combination effects of saquinavir with the nucleoside analogues, AZT, ddC and ddI. The assay used was syncytial cell reduction in CEM cells infected with HIV-1 strain IIIB and cytotoxicity was determined in uninfected CEM cells by the MTT assay. The combination effect was determined by isobologram plots and combination indices.

Results of the drug combination studies suggest that the antiviral effect of saquinavir in combination with AZT, ddC, ddI or *tat* antagonist Ro 24-7429 was synergistic. In these combinations there was no enhancement of cytotoxicity in the concentration range of 1-200 μM. Triple combination of saquinavir + AZT + ddC was also synergistic without enhanced cytotoxicity. The drug combination studies were limited to a single cell line and a single endpoint. The conclusion that the combinations were synergistic drawn by this single cell system must be interpreted with caution because the antiviral effects were based on a series of calculations and extrapolations in this single cell line.

Cytotoxicity of saquinavir: To distinguish the cytotoxic effect from the antiviral effect of saquinavir the sponsor determined the cytotoxic concentration (ID_{50}) of saquinavir against a variety of cell lines to indicate the specificity of saquinavir and calculate therapeutic index. The results of these cytotoxicity studies are presented in Tables 8.1 and 8.2.

Table 8.1. Summary of saquinavir cytotoxicity to human and other primate cell lines

Cell Line	Cell Type	Assay Parameter	Exposure in days	Inhibition
CEM-T4	T lymphoblastoid	MTT[1]	3	11 μM*
		MTT[2]	6	(47.5% at 10 μM)+
		MTT[3]	6	(34.7% at 10 μM)+
		MTT[4]	4	(0% at 10 μM)+
CEM	T lymphoblastoid	Viable cell count	6	10 μM*
H9	T lymphoblastoid	Viable cell count	6	39.4 μM*
JM	T lymphoblastoid	MTT	3	(0% at 100 μM)+
U937	Promonocytic	MTT	3	43 μM*
PBMC	Primary cell culture	^{3}H-thymidine uptake	1	31.6 μM*
C8166	T lymphoblastoid	MTT	3	93 μM*
C8166	T lymphoblastoid	MTT	4	90 μM*

+Inhibition at the highest concentration used given in parenthesis

* minimum inhibitory concentration for 90% colony reduction

1-4. Separate experiments

Table 8.2. Human and other primate cell lines

Cell Line	Cell Type	Assay Parameter	Exposure in days	Inhibition
MT4	T lymphoblastoid	MTT[1]	6	(13% at 10 μM)+
		MTT[2]	6	(0% at 10 μM)+
AA2	B lymphoblastoid	MTT	3	(0%at 10 μM)*
HeLa	Cervical epithelial	Colony Formation	7	(MIC_{50} 37 μM)
HEL	Embryonic lung	Colony Formation	7	(MIC_{50} 73 μM)
HL-60	Promyelocytic leukaemia-derived	^{3}H-thymidine uptake	1	34.8 μM*
SK-N-SH	Neuroblastoma	Colony formation	7	(MIC_{50} 19 μM)
Vero	African Green Monkey Kidney cell line	Viable cell count	3	17.7 μM*

* 50% cytotoxic dose (TD_{50})

+ Inhibition at highest concentration used given in parenthesis

++ Minimum inhibitory concentration for 90% colony reduction

1,2 = separate experiments

Table 9 shows a summary of saquinavir cytotoxicity in cell lines chronically infected with HIV. In all of these assays cytotoxicity was determined by counting the viable cells either by the MTT assay or by the trypan blue exclusion method

Table 9. summary of cytotoxicity in cell lines chronically infected with HIV

Host cell/virus strain combination	Exposure	Inhibition
CEM cells/HIV-1_{IIIb}	3	(0% at 10 μM)+
CEM cells/HIV-1_{IIIB}	2	(49% at 10 μM)+
CEM cells/HIV-1_{IIIb}	3	(0% at 10 μM)+
JM cells/HIV-1_{GB8}	3	0.64 μM"
H9 cells / HIV-2_{ROD}	2	(6.3% at 10 μM)+
U937 cells/HIV-1	3	(0% at 1 μM)+
U937 cells/HIV-1	3	(0% at 1 μM)+
U937 cells/HIV-1	3	(0% at 1 μM)+
U937 cells/HIV-1	3	(20% at 1 μM)+

" 50% cytotoxic dose (TD_{50})

+ Percent inhibition at highest concentration used given in parenthesis

The combined results in Tables 8.1, 8.2 and 9 show that cytotoxicity of saquinavir was observed only at micromolar concentrations while the previously described data show that the antiviral activity was evident at nanomolar concentrations. Comparison of antiviral activities (Table 2-5) and cytotoxicities (Tables 8.1, 8.2 and 9) show that although there is some variation depending upon the host cell system used, there is an overall differential of at least 3 orders of magnitude between antiviral and cytotoxic effects. This favorable therapeutic index is presumed to translate into weak adverse effects, if any, in treated patients

DRUG RESISTANCE: The rate of emergence of resistance depends on several factors which include the rate of mutation per round of virus replication, the number of replications per unit time and the advantage or disadvantage of the mutation for the virus. The replication of HIV is remarkably inaccurate by virtue of it being an RNA virus, because of the greater infidelity of DNA synthesis mediated by the viral RT and by lack of associated proof-reading exonuclease

activity that is generally found in DNA polymerases. The higher rates of replication errors (10^{-4}) in HIV result in the production of progeny virions, of which the genomic RNA of each is molecularly different from each other. Combination of this inherent variability and prolonged treatment with antiviral agents (which are virustatic rather than virucidal and therefore incomplete suppressor of virus replication) results in the emergence and selection of HIV with reduced susceptibility to anti-HIV agents. The prediction of the emergence of resistance to anti-HIV drug has been borne out by experience with the use of any of the clinically available anti-HIV drugs. The finite therapeutic effectiveness of the anti-HIV drugs has been attributed to the emergence of resistance in HIV against these drugs.

Early in vitro experiments with anti-HIV drugs have been predictive of the potential for the emergence of resistance in HIV to the test drug. To explore the possible emergence of in vitro resistance to saquinavir, the sponsor attempted to select for saquinavir resistant variants and characterize them for decrease in the saquinavir susceptibility (phenotypic resistance) and the genetic basis of the decrease in saquinavir sensitivity (genotypic resistance). The resistance analysis has been extended to in vivo studies using patients samples to determine the extent and nature of the clinical resistance. Both in vitro and in vivo resistance studies submitted by the sponsor are reviewed under separate subheadings below

Phenotypic resistance: The sponsor investigated the potential for loss susceptibility in HIV to saquinavir by serial passage of the virus in the presence of increasing concentrations of saquinavir. In parallel, as controls, the nucleoside analogue AZT and the nonnucleoside analogue R82150, were included for comparison. HIV from the serial passages was then titrated for test drug susceptibility.

The host cell/virus strain/endpoint assay system used for selecting virus with reduced sensitivity to saquinavir was CEM/HIV-1_{IIIB}/syncytium assay or p24 antigen determination. In all instances a significant increase in the concentration of the compounds required for inhibition of syncytium formation was found after 7-9 passages Table 10 shows the loss of sensitivity profile of passage 11 virus and lack of cross resistance by the comparator compounds. The drug concentration unit in these studies is nM.

Table 10. Lack of cross-resistance between saquinavir, ZDV and R82150 using in vitro-passaged HIV-1_{GB8} isolate selected against these agents

Relative Sensitivity	Resistance generated to					
	Saquinavir		ZDV		R82150	
	IC_{50} R/WT	IC_{90} R/WT	IC_{50} R/WT	IC_{90} R/WT	IC_{50} R/WT	IC_{90} R/WT
saquinavir	82	39	0.65	1.0	1.2	0.6
R82150	1.2	0.4	0.9	0.4	470	53
ZDV	1.1	2.2	65	175	0.2	3.8
ddC	0.6	1.0	0.75	0.7	1.5	1.1

IC_{50}R/WT = Ratio of resistant/wild type IC_{50}
IC_{90}R/WT = Ratio of resistant/wild type IC_{90}

The results in the Table 10 show that there was a significant increase in the concentration of the test drugs required to attain a similar percent of inhibition. In the case of saquinavir there was an 82 and 39 fold increase in the IC_{50} and IC_{90} concentrations, respectively. The IC_{50} and IC_{90} for the parental virus was 7 and 16 nM, respectively. The results also show that resistance developed to each of the test drug independently and there was no cross-resistance among the three test drugs. Analysis for growth kinetics and durability of resistance indicated that the growth kinetics of saquinavir resistant virus was comparable to the parental virus and the resistance appeared to be stable in the absence of the drug, suggesting alterations in the protease have no effect on the replication of the virus. The result is consistent with the observation of the remarkable ability of HIV protease to mutate and yet preserve function

The sponsor claims that the emergence of resistance to saquinavir was slower and of a lower degree than with the representative RT inhibitors tested. The extrapolation to other drugs often is not appropriate since the intracellular levels of activation enzymes and half-lives of 5'-phosphorylated derivatives are not known. The relative ease or time taken to achieve the in vitro

selection of resistant variants cannot be used as a measure of the in vivo situation. However, these data may be useful in predicting the likelihood of HIV-1 drug resistance in the clinic.

Genotypic resistance: Prior experience with nucleoside and non-nucleoside analogue inhibitors of HIV-RT showed that the molecular basis of *in vitro* resistance was due to missense mutations in the RT coding sequences and this mutation pattern to a large extent is predictive of the mutation pattern observed in the patients during treatment. The sponsor investigated the genetic basis for in vitro resistance of the protease inhibitor, saquinavir, which may also be predictive of in vivo resistance to the drug.

HIV variants with reduced susceptibility to saquinavir were selected by repeated passage of the parental virus in CEM cells in the presence of increasing concentrations of the inhibitor. The proviral DNA corresponding to the protease in CEM cells was sequenced and compared to the sequence of the parental virus. Sequencing results showed that two amino acid substitutions in the protease codons of the resistant virus; a glycine to valine exchange at position 48 and a leucine to methionine exchange at position 90. The Gly 48 Val mutation has emerged singly or together with Leu 90 Met in saquinavir resistant protease sequences derived from laboratory passaged HIV-1_{IIIB}, HIV-1_{MPVWW} and HIV-1_{MPV1A}. The independent resistance discovered in several strains of HIV-1 imply an important role for Gal 48 Val and Leu 90 Met mutations in reducing sensitivity to saquinavir. On some occasions during the in vitro passage two other mutations at Ala 71 Thr and Ile 84 Val have occurred and the significance of these mutations is unknown.

The biological significance of the amino acid changes at position 48 and 90 was confirmed by construction of recombinant viruses containing the substitutions at these positions and subsequent testing of their susceptibilities to saquinavir. Table 11 shows the sensitivity of HIV-1 HXB2 wild type virus and recombinant virus containing protease mutations to saquinavir. The results indicate that the recombinant mutant viruses containing single mutations at positions 48 showed an 8-fold and that at 90 showed a 3.4-fold resistance to saquinavir with concomitant increase in the IC_{90} values. Recombinant mutant viruses containing both the mutations, however, showed a 20-fold decrease in saquinavir susceptibility. Growth kinetics of these resistant viruses were comparable to the parental viruses and the resistant genotype was stable in the absence of the inhibitor.

Table 11. Sensitivity of HIV-1 HXB2 wild-type virus and proteinase mutants to saquinavir

Virus	Host Cells	IC_{50}*	IC_{90}*
HXB2 $_{wt}$	MT-2 PBMC	1.7 nM 6.0 nM	8.9 nM n.d.
HXB2 G48V	MT-2	13.5 nM	20.0 nM
HXB2 L90M	MT-2	5.7 nM	15.1 nM
HXB2 G48V+L90M	PBMC	120 nM	n.d.

*IC_{50}, IC_{90}: 50%, 90% inhibitory concentrations of saquinavir
n.d.: not determined

The evolution of resistance to saquinavir initially seems to involve a selection of rare, or perhaps pre-existing wild-type genotype. Sequencing of intermediate passaged HIV-1_{GBA} virus showed mutations at positions 36, 57 and 63 (Table 12), suggesting other amino acid contributions in early steps of progressive changes in the development of resistance.

Table 12. Genotype of selected HIV-1_{GBA} variants and sensitivity of saquinavir

Passage number	Amino acid identity at positions					IC_{90} (nM)
	36	48	57	63	90	
0	M/I	G	K/R	L/V	L	25
7	I	G	R	V	L	34
8	I	V	R	V	L	360
11	I	V	R	V	M	700

The importance of the changes at 36, 57 and 63 in early passages for the selection of HIV-1_{GS} remains unclear since the IC_{90} value is slightly higher than that of the parental virus. It is conceivable that residues at these positions could compensate for detrimental effects of the position 48 and 90 mutations on the protease activity and fitness of the virus.

Viruses containing the double mutations at positions 48 and 90 are capable of growing in the concentration range of 1000-1500 nM. Such concentrations at the proposed dose of saquinavir (600 mg TID) are not attainable in the plasma, suggests loss of antiviral efficacy when these mutations occur in the clinic. The data also suggests that it is not necessary for the virus to undergo both mutations to be able to replicate in the treated patients at the proposed dose and therefore is less likely to develop and retain both mutations under conditions of the current dose regimen. This prediction is consistent with the sponsors observations that in clinical samples only 3 out of the 38 mutant viruses contain the double mutation. A single mutation at either site (amino acid positions 48 or 90) should be sufficient to bypass the inhibitory effect of saquinavir as the average concentration under the dosage conditions is 3 times over the IC_{90} value.

Clinical Resistance: In a subset of HIV patients treated with saquinavir the sponsor tested the virus both for reduced HIV susceptibility (phenotypic resistance) to saquinavir and the genetic basis for the reduced susceptibility (genotypic resistance). The phenotypic and genotypic resistance analysis was not always done with matched virus samples. The sponsor's assumed definition for phenotypic change was a 10-fold or greater relative increase in IC_{90} with respect to individual patient's baseline value. A genotypic change was defined by either the G48V or L90M mutation or both in the protease gene. The protease gene sequenced for defining the resistance mutations was the integrated proviral DNA extracted from the patients PBMC. The samples were collected between 16-74 weeks of therapy (median observation period of approximately 1 year) and the resistance data is summarized in Table 13. In different clinical trials a total of 39 patient samples were analyzed for phenotypic changes and 85 samples for genotypic changes. The development of resistance in monotherapy and combination therapy are presented in table 13.

Table 13. Frequency of genotypic and phenotypic changes in selected patients treated with saquinavir.

Treatment	Phenotypic change	Genotypic change
Monotherapy	5/11 (45%)	15/33 (45%)
Combination therapy*	11/29 (38%)	16/52 (31%)

*include both double (saquinavir + AZT) and triple combination (saquinavir + AZT + ddC)

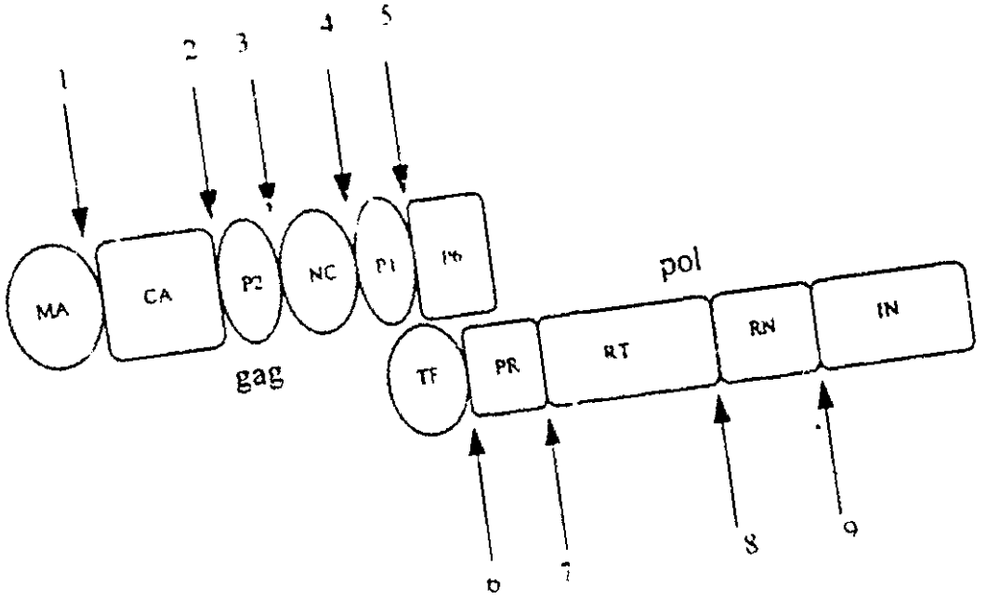
The resistance data of this submission should be interpreted with caution since the sponsor's assessment of decrease in HIV susceptibility and its genetic basis suffers from multiple deficiencies. For example, the pharmacokinetic results indicate that the average plasma concentration of saquinavir was 3 fold the IC_{50} value even if all of the 98 % protein bound drug is available for antiviral activity and yet in the determination of phenotypic resistance the sponsor chose an arbitrarily high (10-fold the IC_{50} value) threshold concentration of saquinavir to be scored as positive in the assay. In the analysis for the genetic basis for the resistance, protease gene from the integrated proviral DNA of PBMC was used. It is recognized that genotypic changes occur in the viral RNA earlier than in the PBMC DNA due to low penetrance of viral DNA into PBMC.

Cross-resistance: For effective anti-HIV therapy, combination of agents that employ distinct modes of action and having non-overlapping resistance profiles offer greater therapeutic advantage over single agents or single target site therapies. The proposed combination of RT inhibitors and protease inhibitor which mediate antiviral activity through two independent mechanisms on two different viral targets at two different stages of the virus replication cycle showed prolonged antiviral suppression than monotherapy with saquinavir. However, resistance is still likely to develop, albeit slowly, and if the resistance profile is non-overlapping then that could translate into prolonged anti-HIV activity and delayed disease progression. As anticipated in vitro combination studies indicate no cross-resistance between the protease inhibitor saquinavir and RT inhibitors in either direction because of the different targets involved.

Many protease inhibitors are currently undergoing clinical trials and several of them may be available for clinical use in the near future. Theoretical considerations predict cross-resistance among peptidomimetic inhibitors of HIV protease. For appropriate future combination clinical use, it is of interest to determine if the saquinavir-resistant variants also express cross-resistance to other experimental protease inhibitors. Published literature indicates that comparison of mutant profiles with other experimental protease inhibitors prompted a different pattern of mutations that are infrequently expressed in patient treated with saquinavir. However, resistance mutation profiles expressed with experimental protease inhibitors such as Merck's inhibitor, indinavir, showed different degree of cross-resistance to other protease inhibitors. Cross-resistance studies among protease inhibitors are in the early stages of exploration and as these studies mature in the future, proper scientific and treatment decisions could be made. Cross-resistance studies are also complicated by the low plasma concentrations of saquinavir which may elicit a different set of resistant mutation profiles than under conditions of high plasma concentration with potential differences in cross-resistance. The general conclusion from the rapidly changing cross-resistance patterns is that cross-resistance exists among the protease inhibitors, albeit to different degree, and may not support combination use of these drugs.

MECHANISM OF ACTION: An essential step in HIV life cycle involves the specific cleavage of viral structural polyprotein precursor *gag* ($p55^{gag}$) and enzyme polyprotein precursor *gag-pol* ($p160^{gag\text{-}pol}$) into their mature functional forms. The specific cleavages in the naive non-infectious virions are carried out by the HIV encoded protease resulting in the formation of mature infectious HIV. Based on the structure and mechanism of action, the HIV protease has been classified as a member of the aspartyl protease family of enzymes and is related to cellular aspartic proteases. However, the cellular proteases cannot substitute for HIV protease function. This indispensable role of the HIV protease for the production of infectious virus was demonstrated by genetic and biochemical experiments. Genetic experiments by site-directed mutations involving the active site amino acids of the protease resulted in the production of noninfectious virus. Similarly, complementary genetic studies involving mutations in the cognate cleavage site amino acids on the *gag* and *gag-pol* substrates also resulted in the production of non-infectious HIV. The protease cleaves at least 9 distinct sites on the *gag* and *gag-pol* proteins and the processing sites are diagrammatically shown in the Figure 1

Figure 1. HIV protease cleavage sites on *gag* and *gag-pol* polyproteins



1: Gln. Asn. Tyr * Pro. Ile Val
2: Arg. Val. Leu * Ala. Glu. Ala
3: Thr. Ile. Met * Met. Gln. Arg
4: Gln. Ala. Asn * Phe. Leu. Gly
5: Gly. Asn. Phe * Leu. Gln. Ser
6: Phe. Ser. Phe * Pro. Gln. Ile
7: Leu. Asn. Phe * Pro. Ile. Ser
8: Glu. Thr. Phe * Tyr. Val. Asp
9: Lys. Val. Leu * Phe. Leu. Asp

* indicates cleavage site

The abbreviations are for proteins of MA, matrix protein; CA, capsid protein; NC, nucleocapsid protein; TF, transframe protein, PR, proteinase; RT, reverse transcriptase; RN, RNase H; IN, integrase

Saquinavir is a rationally designed non-hydrolyzable peptidomimetic inhibitor of the HIV protease. The substrate based selection of the inhibitor is facilitated by a wealth of data on the protease crystal structure of both native enzyme and enzyme inhibitor complexes and extensive biochemical data detailing catalytic mechanism of protease action. To obtain low K_i value and derive maximum inhibitory benefit the sponsor optimized saquinavir to mimic the configuration between the aromatic acids and proline namely Phe/Tyr-pro cleavage site, because three of the processing sites at 1,6 and 7 (see Fig.1) on the polyprotein substrates have this structure.

Saquinavir has been reported to competitively inhibit HIV type 1 and type 2 protease. The kinetic assays were performed using purified recombinant protease expressed in bacteria. The peptide substrate was synthetic and natural polyprotein substrates were expressed in a baculovirus expression system. The sponsor's data on the antiviral activity of saquinavir by biochemical evidence showing inhibition of polyprotein processing, morphological evidence indicating the production of immature virions by electron microscopy and the biological evidence showing lack of infectivity of the morphologically immature virions are consistent with the proposed mechanism of action of saquinavir as an inhibitor of HIV protease activity.

CONCLUSIONS:

HIV protease is an indispensable virus-coded enzyme required to proteolytically cleave the long polyprotein precursors of the HIV *gag* and *pol* gene products. The cleavage of the polyprotein precursors in the budding HIV virions results in the transformation of the immature non-infectious HIV into mature infectious HIV. Thus, the protease function is a late stage function in the virus replication cycle and constitutes "an extracellular" event. The sponsor presented data which indicates that saquinavir inhibits HIV protease and as a consequence blocks the sequence of events starting with the inhibition of cleavage of polyproteins and ending with the packaging of immature non-infectious virions which are unable to initiate new rounds of infection. Some of the data that saquinavir inhibits HIV protease leading to improper assembly of the HIV virions include: (1) biochemical evidence by immunoblot analysis which show that the virion polyproteins fail to be processed into their functional proteins in the presence of saquinavir, (2) morphological evidence by electron microscopy which indicates that the virions formed in the presence of saquinavir are immature, (3) biological evidence which showed lack of infectivity of the morphologically

immature virions by titration of the virions for infectivity and (4) evidence from anti-HIV data generated by titration for multiple endpoints which show that saquinavir inhibits virus production in different cell types infected with a variety of HIV variants. All of the experimental evidence is consistent with the proposed mechanism of action that saquinavir is an inhibitor of HIV protease activity and as a consequence produces non-infectious HIV that is unable to initiate new rounds of infection.

Some of the antiviral activity measures used suffer from pitfalls. The function of HIV protease is to ensure the correct processing of viral structural proteins and enzymes (and not the production of the virus itself). The processed proteins in association with the viral genomic RNA organize into mature infectious HIV. Quantification of the viral structural protein p24 by immunoassay and the viral RNA by PCR do not distinguish between the naive immature noninfectious virions generated as a consequence of saquinavir treatment from the mature infectious virions formed in the absence of the inhibitor. (In the p24 immunoassay the p24 antibodies used recognize the epitopes both on its precursor polyprotein, p55 in noninfectious virus, and the product p24 in the infectious virus and does not distinguish between the two forms). Similarly, quantification of viral RNA by PCR fails to distinguish the RNA of the infectious virus from the noninfectious virus. However, the combination anti-HIV data derived from multiple methods of analysis support the antiviral activity of saquinavir

The proposed dose of saquinavir is suboptimal for sustained anti-HIV activity. In vitro antiviral activity data derived from a variety of host cells-virus strain combination studies including primary cells infected with clinical isolates of HIV, show that the average IC_{50} and IC_{90} concentrations of saquinavir is 20 and 100 nM, respectively. Clinical Pharmacokinetic studies indicate that at steady state the plasma concentration of saquinavir is approximately 80 ng/ml or 61 nM. Of this 98% of the drug is protein bound and therefore the free concentration of 2% corresponds to 1.6 ng/ml. This low plasma concentration is insufficient to completely block HIV maturation. Extrapolation of the PK data to the anti-HIV activity suggests low levels of inhibition and is consistent with the poor efficacy of saquinavir in monotherapy and improved efficacy in combination with nucleoside analogues.

Saquinavir is an incomplete inhibitor of HIV protease. The viral protease is a highly flexible molecule with an ability to recognize many distinct peptide sequences and cleaves at as many

as 12 different sites in a specific and orderly manner. Although saquinavir by design was optimized to interfere with a minority of three "shared substrate processing sites" for the protease, it was not designed to interfere with the majority of approximately nine "unshared substrate processing sites" of the protease. However, such incomplete inhibitors could still be functionally effective by completely blocking a single processing site on the polyprotein substrates and thus prevent the production of one or two essential components of the full complement of the enzymes and/or structural proteins required for the retention of viral infectivity.

Saquinavir offers multiple benefits over nucleoside analogues. Unlike the nucleoside analogue prodrugs which require prior metabolic activation by the host cell enzymes, the protease inhibitor, saquinavir, is a direct acting drug and therefore, should be active in all cell populations regardless of the metabolic state of the host cells. Consistent with this fact it was observed that saquinavir was active in mitotically active cells such as lymphoid cells, cells deficient in nucleotide metabolizing enzymes like JM cells and terminally differentiated cells like monocyte-macrophages. Saquinavir as an inhibitor of protease activity also blocks virus production in chronic and acute infections unlike nucleoside analogues whose activity is limited to acute infection. Saquinavir as a rationally designed drug is specific to the protease and therefore is less likely to elicit toxicities, at least not on mechanistic grounds. Mechanistic toxicity of nucleoside analogues to cellular DNA polymerases and consequential side effects in treated individuals are well recognized. Therefore, under pharmacokinetically balanced dose conditions, monotherapy with saquinavir should provide greater viral suppression and consequential benefits than monotherapy with nucleoside analogues

Combination therapy with saquinavir and RT inhibitors should be additive to synergistic. The rationale for combination therapy with anti-HIV agents is to enhance viral suppression, decrease toxicities and limit the emergence of drug resistance. Combination of an RT inhibitor like AZT which is effective in the initial stages of HIV infection inside the infected cells and the protease inhibitors like saquinavir which is effective in the terminal stages of viral production outside the infected cells, should, in addition to being additive to synergistic, also be effective in different cell populations. The enhanced and more sustained viral suppression and slower emergence of resistance reported in in vitro and in in vivo data using this combination is consistent with two antiviral agents which exert antiviral activity through two independent mechanisms on two different viral targets at two different stages of the virus replication and thus fulfill some of the

expectations of combination therapy. Triple drug combinations particularly those involving mutation suppressing agents (AZT+3TC combination) with protease inhibitor may provide additional benefit by further delaying resistance. Furthermore, in multiple drug combinations HIV may be constrained from developing combination of mutations required for multidrug resistance although there appears to be no "genetic barrier" for multidrug resistance in HIV.

In vitro saquinavir resistance is correlated with resistance in the clinic. Multiple studies with RT inhibitors, and accumulating data from experimental protease inhibitors including saquinavir indicate that phenotypic and genotypic resistance develops in vitro against each of these drugs. The in vitro mutation pattern engendering resistance was also reflected in the HIV recovered from the clinical samples of treated patients. The early in vitro resistance studies, therefore, have been predictive of the likelihood of HIV resistance in the clinic and thus greatly help in the drug development decisions of experimental HIV therapies. Analysis for the molecular basis of saquinavir resistance of both *in vitro and in vivo derived* HIV isolates showed that the mutations elicited in the protease gene were the same and that involved amino acid positions 48, 90 or both. The number and types of amino acid changes in the HIV protease appear to be those that will generate just sufficient level of resistance to bypass the drug pressure. In the case of saquinavir the average plasma concentration in patients is about 4-fold over the IC_{50} value and the predominant amino acid change detected was Leu 90 Met, which extends about a 4-fold loss of drug susceptibility which is enough to bypass the drug pressure. In a minority of patients the Gly 48 Val mutation which extends about 8-fold loss of susceptibility occurred and the double substitution which extends 20-fold resistance occurred rarely (in 2 out of 85 patients). The mutations pattern observed in saquinavir treated patients is consistent with the hypothesis that the magnitude of decrease in susceptibility depends on the concentration of the inhibitor available in the virion environment and the virus undergoes those changes sufficient to bypass the existing drug concentration.

Clinical resistance is progressive with higher levels of resistance associated with greater number of mutations. Published reports show that the 99 amino acid peptide of HIV protease can substitute as many as 20 amino acids resulting in >1000-fold reduction in drug susceptibility. *The amino acid changes result in reduced affinity to the inhibitor. In spite of extensive changes* in the peptide the enzyme continues to preserve its function. Available data from investigational protease inhibitors and saquinavir indicate that resistance expression resulted by multiple co-

expression of substitutions at several protease sites and greater degree of resistance appeared to be co-related with the co-expression of greater number of amino acid substitutions. Thus, the HIV protease endowed with the remarkable ability to mutate rapidly without loss of protease activity appears to be a faster moving target for attack with protease inhibitor monotherapy. Combination therapy, particularly those involving different molecular targets on the virus, for reasons stated earlier may be a more appropriate approach to attain a more sustained viral suppression and slower emergence of resistance, although no "genetic barrier" appears to exist for multidrug resistance.

Multidrug treatment approach with combination use of protease inhibitors is premature. Most of the current experimental drugs that target the HIV protease are substrate-based, non-hydrolyzable inhibitors that initially target the wild type protease. These inhibitors are selected for their ability to bind tightly and inhibit the protease activity. The strength of binding is determined by their low K_i value. Mutating any of the amino acid residues that modify substrate binding pockets alters the favorable interaction between the enzyme and inhibitor and the K_i for the 'class' of inhibitors increases. The magnitude of increase in the K_i could be dramatic and depends on the concentration of the inhibitor available in the virion environment. Cross-resistance among substrate-based non-hydrolyzable inhibitors is likely and consistent with this prediction are the recent reports in the literature which indicate varying degrees of cross-resistance among protease inhibitors. For example, complete cross-resistance between Crixivan® and Ritonavir®, in both directions, and variable degrees of resistance with other inhibitors have been reported. To date at least 20 different amino acid substitutions in the 99 amino acid protease have been reported and attests to the remarkable ability and flexibility of the enzyme to mutate and yet preserve its function. Therefore, caution is suggested for multidrug treatment approach with combination use of protease inhibitors.

Phase 4 considerations:

1. Please reduce the current threshold of 10-fold in loss of saquinavir susceptibility for positive scoring as phenotypic resistance. Clinical Pharmacokinetic studies indicate that at steady state the plasma concentration of saquinavir is approximately 80 ng/ml or 61 nM. Of this 98% of the drug is protein bound and therefore, the free concentration of 2% corresponds to 1.6 ng/ml. The plasma saquinavir concentration corresponds to < 5-fold the IC_{50} value even if we assume that all of the protein-bound drug is available for

antiviral activity. Thus, under the current dosing conditions the arbitrary 10-fold cut off for phenotypic resistance is too high a concentration for scoring in the assay.

2. Please use circulating viral RNA for determination of the molecular basis of genotypic resistance by sequencing the entire protease coding domain. In the NDA submission, genotypic changes were scored by sequencing the protease gene in patients PBMC. Evidence in the literature indicates that genotypic changes occur in the viral RNA earlier than in the PBMC due to low penetrance of the HIV variants into the PBMC. Therefore, sequencing of the protease codons in the viral RNA rather than in the proviral DNA is appropriate for determining genotypic resistance.

RECOMMENDATIONS: The microbiology portion of the draft label as currently written is acceptable. With respect to microbiology the NDA is approved.

Narayana Battula, Ph.D.
Microbiologist

CONCURRENCES:
HFD-530/Deputy Dir _____ Signature _____ Date
HFD-530/SMicro _____ Signature _____ Date
CC:
HFD-530/Original IND
HFD-530/Division File
HFD-530/MO
HFD-530/Pharm
HFD-530/Chem
HFD-530/SMicro
HFD-530/Review Micro
HFD-530/CSO, Kinsey, V

CLINICAL PHARMACOLOGY: ***Mechanism of Action*** HIV protease cleaves viral polyprotein precursors to generate functional proteins in HIV-infected cells. The cleavage of viral polyprotein precursors is essential for maturation of infectious virus. Saquinavir mesylate, *hencefortli referred to as saquinavir*, is a synthetic peptide-like substrate analogue that inhibits the activity of HIV protease and prevents the cleavage of viral polyproteins.

Microbiology: *Antiviral Activity In Vitro* The 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 values (50% inhibitory concentration) were in the range of 1 to 30 nM. In cell culture saquinavir demonstrated additive to synergistic effects against HIV in double and triple combination regimens with reverse transcriptase inhibitors zidovudine (ZDV), zalcitabine (ddC) and didanosine (ddI), without enhanced cytotoxicity.

Resistance: HIV isolates with reduced susceptibility to saquinavir have been selected in vitro. Genotypic analyses of these isolates showed substitution mutations in the HIV protease at amino acid positions 48 (Glycine to Valine) and 90 (Leucine to Methionine).

Phenotypic and genotypic changes in HIV isolates from patients treated with saquinavir were also monitored in Phase 1/2 clinical trials. Phenotypic changes were defined as a tenfold decrease in sensitivity from baseline. Two viral protease mutations (L90M and/or G48V; the former predominating) were found in virus from treated, but not untreated, patients. The incidence across studies of phenotypic and genotypic changes in the subsets of patients studied for a period of 16 to 74 weeks (median observation time approximately 1 year) is shown in Table 1. However, the clinical relevance of phenotypic and genotypic changes associated with saquinavir therapy has not been established.

Table 1. Frequency of Genotypic and Phenotypic Changes in Selected Patients Treated with Saquinavir

	Genotypic*	Phenotypic†
Monotherapy	15/33 (45%)	5/11 (45%)
Combination Therapy	16/52 (31%)	11/29 (38%)

* Double mutation (G48V and L90M) has occurred in 2 of 33 patients receiving monotherapy.
† For some patients genotypic and phenotypic changes were unrelated.

Cross-resistance to Other Antiretrovirals The potential for HIV cross-resistance between protease inhibitors has not been fully explored. Therefore, it is unknown what effect saquinavir therapy will have on the activity of subsequent protease inhibitors. Cross-resistance between saquinavir and reverse transcriptase inhibitors is unlikely because of the different enzyme targets involved. ZDV-resistant HIV isolates have been shown to be sensitive to saquinavir in vitro.

PHARMACOLOGIST'S REVIEW

NDA 20-628

Original NDA and amendments (BP, NC)
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Date Assigned: 9/5/95
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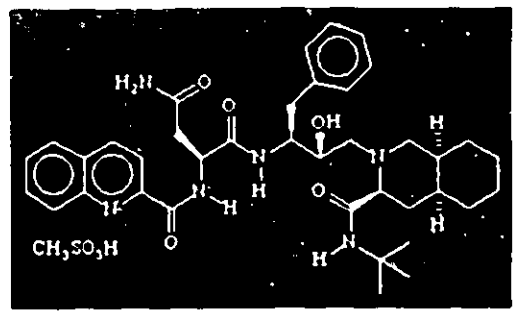
Reviewed by: Kuei-Meng Wu
HFD-530

SPONSOR:

Hoffmann-La Roche Inc
340 Kingsland Street
Nutley, New Jersey 07110

DRUG:



INVIRASE®, Saquinavir, Ro 31-8959/003, cis-N-tert-Butyldecahydro-2-[2(R)-hydroxy-4-phenyl-3(S)-[[N-(2-quinolylcarbonyl)-L-asparaginyl]amino]butyl](4aS,8aS)-isoquinoline-3(S)-carboxamide methylsulfonate. Formula: $C_{38}H_{50}N_6O_5.1:1CH_4O_3S$; MW: 767 (free base = 671)

FORMULATION: Gelatine capsule 200 mg (as free base) (Inactive ingredients: lactose, microcrystalline cellulose, povidone K30, sodium starch glycolate, talc and magnesium stearate)

INDICATIONS: Monotherapy and Combination Treatment (with HIVID and ZDV) for Patients with Advanced HIV Infections

INTRODUCTION

This NDA (saquinavir) emanated from IND that was originally submitted on 11/23/1992. Through a successful three and half years preclinical and clinical development program, the sponsor is seeking for approval of saquinavir for the treatment of HIV infections at an oral dose of 600 mg tid. The efficacy is based on data collected from a total of 920 intent-to-treat HIV + patients that showed greater increases in CD_4 count and reductions of viral RNA copies with combination therapyies including saquinavir, than corresponding treatments without saquinavir. The preclinical program of this NDA consisted of more than 45 animal toxicity studies conducted in rodents, marmosets, dogs, and rabbits in support of the intended human uses. Most of the toxicity studies have been reviewed under IND. This review document summarizes and comments on the up-to-date preclinical safety information and the proposed labeling on saquinavir.

BACKGROUND

The viral proteinase plays a vital role in the production of a number of essential proteins during the later stages of the replication of HIV. The enzyme is one of the "aspartic acid" family of proteinases, similar to the human enzymes renin and pepsin but with a particular

ability to catalyze the hydrolysis of peptides adjacent to prolyl residues. Saquinavir is an analogue of a typical substrate for the HIV proteinase and contains a hydroxyethylamine transition state mimetic of the phenyl-prolyl peptide bond. It is a highly potent inhibitor of the enzymes from both HIV-1 and HIV-2 and exhibits K_i values at approximately 10^{-10}M. In the in vitro models, saquinavir has potent antiviral activity against both HIV-1 and HIV-2 and prevents the maturation of the viruses in chronically infected cells. Contrasting to its potent inhibitory effect on HIV proteinase, saquinavir has little activity against human aspartic, serine, cysteine, and metallic proteinases.

SUMMARY OF PRECLINICAL SAFETY INFORMATION: SAQUINAVIR (RO 31-8959)

Oral and iv toxicity and toxicokinetic studies on saquinavir in animals (mice, rats, marmosets, dogs, rabbits) have uncovered a moderate toxicity profile at plasma exposure levels higher than those seen in the human trials. The data would be employed to support the drug to be used in adult patients via the oral route. For a detailed review on the animal toxicology and pharmacology studies, please refer to the SECTION I and II of APPENDIX (p. 8-62) of this document. Key preclinical safety information is recapitulated and issues discussed below.

(1) TARGET ORGAN/SYSTEM AND PROFILE OF TOXICITY

The sponsor has completed a series of repeat-dose toxicity studies in rats, marmosets and dogs. The maximal study duration reached to 6 months in the rat and marmoset. The potential target organ/system of toxicity explored from the repeat-dose studies are highlighted below:

Liver Both rat, dog and marmoset studies on saquinavir showed signs of liver toxicity. In the oral studies conducted in rats, increases in plasma transferase enzymes (AST and ALT) were observed at 400 mg/kg/day or higher (13-week and 6-month study) whereas there was no evidence of histological change in the liver of these animals. The plasma exposure (AUC_{24h}) of HIV patients administered the standard clinical dose of 600 mg saquinavir tid is approximately 2000 ng×h/ml with a maximal plasma concentration (C_{max}) of approximately 240 ng/mL. Plasma exposure to saquinavir in the rat at the 400 mg/kg/day dose ($C_{max} \approx 700$ ng/ml; $AUC_{24h} \approx 6000$ ng×h/ml) was approximately 3-fold greater than both the C_{max} and AUC_{24} values predicted in patients In the oral study conducted in dogs, elevations in ALP, ALT, and GGT values, and transient increases in AST, cholesterol, bilirubin, and triglyceride values were seen at 700 mg/kg/day ($AUC_{24h} \approx 177000$ ng×h/ml, 4-week dog study). Histopathology in dogs with aberrantly higher drug exposures showed scattered necrosis of individual hepatocytes, minimal bile duct proliferation, light-brownish pigment in Kupffer cells, and vascular (sinusoidal) leucocytosis. In the ongoing one-year oral study conducted in marmosets, increases in ALP, ALT, and AST values were also reported at 3000 mg/kg/day ($AUC_{24h} \approx 64000$ ng×h/ml, by extrapolation). Abnormal liver enzymes increases were observed in the clinical trials and mentioned in the drug's label under the ADVERSE REACTIONS section

Blood Decreases in the hemoglobin, hematocrit, packed cell volume and red blood cell count (anemia), together with alterations in the number of platelets (generally a marked increase), and increases in leukocytes (primarily neutrophils) were often seen in the repeat-dose studies in rats, occurring at dose levels above 30 mg/kg po or 3 mg/kg iv. These toxicities are generally mild and reversible. No related bone marrow histological changes were observed.

GI Saquinavir caused emesis, and loose stools/diarrhea in dogs given 700 mg/kg po. (exposure levels ≈ 177000 ng×h/ml). Post-dose emesis and diarrhea also occurred in marmosets at 1000 mg/kg po. Diarrhea is the most common side effects seen in the clinical trials with this drug.

Heart In a six-month marmoset study, myocardial fibrosis or myocarditis was reported in 1/6, 0/6, 5/6 and 4/6 of the animals treated with 0, 50, 200 or 750/1000 mg/kg/day of saquinavir, respectively, at the terminal sacrifice. In the recovery animals, these findings were still present in 1/6 each of the 0, 50, 200 mg/kg/day dosage groups. Cardiac fibrosis findings in the heart did not occur in the 13-week mouse study, 4-week/13-week/6-month rat studies, 4-week dog study or 4-week/1-year marmoset studies. No treatment-related ECG changes were reported in both marmoset and dog studies. Because myocardial toxicity findings in the 6-month marmoset study were not seen in studies conducted in other species and were not reproducible in other marmoset studies, the toxicity findings should be considered incidental.

Vascular Irritation Saquinavir is highly irritative to the vein of the rat, marmoset and dog. Chronically implanted venous cannulas in rats had induced irritative injuries in the vein and tissues around the catheterization that, in turn, caused *lethal* damages to the adjacent tissues/organs in the peritoneal and chest cavity (death rate caused by 2-week iv infusions of saquinavir: 0/6, 4/6, 3/6, 5/6 and 6/6 in the groups receiving 0, 100, 300, 600 and 1000 mg/kg/day, respectively). The vascular irritation toxicity of saquinavir may preclude its development for parenteral use.

Neonates Saquinavir is also very irritative to the infantile GI. At a single-dose of 1200 mg/kg, oral saquinavir caused a near 50% deaths of suckling rat pups (administered from day 4 to day 21) (gross examination showed abnormal yellow excretion, red/yellow staining around the anus and inflammation of recto-anal junction/vagina). At a lower dose range (125 and 375 mg/kg/day) the death rate is around 30-40% after 8-18 days of repeat-dosing. The plasma drug levels of these neonates after repeat dosing were in the range of those obtained in the clinical trials (AUC_{ss} ranges = 255-701 ng×h/ml). The cause of death was not known but may be due to saquinavir's irritative potential to the immature GI tract. This information will be important for future pediatric use of the drug.

An oral 12-month toxicity study in marmosets is currently in its recovery phase (in-life phase completed in September, 1995), and additional toxicities observed will be evaluated later when data become available. The sponsor will perform an additional 13-week dog

study to further explore the toxicity profile of the new formulation of this drug.

(2) REPRODUCTIVE TOXICITY

Saquinavir did not produced significant teratology and reproductive toxicities in animals at the doses tested (maximum dose range: 1000 mg-1600 mg/kg).

FERTILITY AND GENERAL REPRODUCTIVE PERFORMANCE (SEGMENT I). In a fertility and general reproductive performance study in rats, no adverse effects were seen at doses up to 1200 mg/kg/day. In this study, toxicokinetic analysis confirmed dose-proportional and continuous (i.e., 24 hour) exposure to saquinavir, with a maximal AUC_{24h} reaching 9700 ng×h/ml (*cp.* HIV+ patients = 2000 ng×h/ml). The development, fertility and reproductive performance of the F_1 generation animals derived from F_0 generation animals were unaffected by F_0 generation treatment. The growth and development to weaning of the untreated F generation were unaffected by F_0 generation treatment at the dose levels used.

TERATOLOGY (SEGMENT II) An embryo toxicity and teratogenicity study in rats at doses up to 1600 mg/kg/day (maximal exposure reached 11000 ng×h/ml) revealed no treatment-related maternal toxicity, embryo toxicity or teratogenicity. Teratogenicity was also evaluated in rabbits at dose levels up to 1000 mg/kg/day. Several incidences of fetal defects in both control and treatment groups occurred. The abnormalities consisted mainly of dilated brain ventricles, folded retinae, closed nasopharyngeal ducts, shortened jaws, spina bifida, ectopic abdominal organs, brachycaudia, fused or bipartite sternebrae together with isolated occurrences of cleft palate, acephaly (aborted fetus), hyperflexed limbs, talipes (aborted fetus), and dichotomy of ribs. The numbers of fetuses and litters with malformations elicited for each dose groups were as follows: 5 fetuses/4 litters (control), 3/3 (100 mg/kg/day), 7/7 (300 mg/kg/day), and 8/7 (1000 mg/kg/day). The findings on defects in 300 and 1000 mg/kg/day groups (drug exposure levels = 3800 and 8000 ng×h/ml, respectively) that showed a higher frequency than concurrent control group were: closed nasopharyngeal ducts, spina bifida, ectopic abdominal organs, brachycaudia, fused sternebrae and cleft palate. However, theses findings failed to be induced in a pilot study using 2000 mg/kg and the incidence was below the sponsor's historical control data. The teratologic potential of Ro 31-8959 in animals can be considered minimal.

DEVELOPMENTAL REPROTOXICITY (SEGMENT III) Administration of saquinavir to rats at doses up to 1600 mg/kg/day during late pregnancy and through lactation (Segment III) had no effects on gestation or parturition nor on the survival, growth and development of the offspring to weaning. The exposure to saquinavir in pregnant rats was confirmed that it was similar to that reported in non-pregnant rats. The placental transfer of radioactive saquinavir to fetuses was minimal (24 ng equivs/g BW).

(3) MUTAGENICITY AND GENOTOXICITY STUDIES

Saquinavir did not show significant mutagenic activities in either bacterial (Ames test) or mammalian cells (V79/HPRT test). It has no clastogenic activity in the mouse micronucleus assay or in human peripheral blood lymphocytes and does not induce primary DNA damage in the unscheduled DNA synthesis test.

(4) CARCINOGENICITY STUDIES

No data from carcinogenicity studies are yet available. A study in mice began in February 1995 in which saquinavir is being incorporated in the diet to give dose levels of 0, 200, 700 and 2500 mg/kg/day. The equivalent dietary admixture study will begin in rats in May 1995 at dose levels of 0, 125, 350 and 1000 mg/kg/day. Both study protocols gained concurrence by the Executive Carcinogenicity Assessment Committee, CDER, FDA.

(5) DRUG METABOLITES AND DRUG INTERACTIONS

Metablite Drug metabolism in animals was quite complex (dose-dependent) and their metabolites were not yet identified (see SECTION III of APPENDIX, p. 63). In rats, at least 7 to 50 metabolites, depending on the dosage, were suggested. The role of drug metabolites in the expression of toxicities seen in various studies is not known.

Drug Interaction No synergistic toxic and toxicokinetic interactions were observed between saquinavir and zidovudine in a four-week oral combination study of these two drugs in the mouse. This information was obtained to support saquinavir's indication for combination use with zidovudine.

(6) RISK ASSESSMENT BASED ON PRECLINICAL TOXICITY DATA

Based on the information summarized above, it is concluded that the toxicity of oral saquinavir is mild. Toxicology tests have employed sufficient dosage and exposure to explore potential adverse effects. The systemic toxicities revealed so far in the animal studies (up to 6 months) are related to liver and hematological system. These toxicities generally occurred at exposure levels several fold (i.e., above 3) higher than in clinical trials, and were considered to be reversible. Clinical trials on saquinavir so far have seen scattered side effects related to liver function and the hematological system. GI irritation such as diarrhea reported in dog and marmoset studies are the major clinical adverse reactions seen with ingestion of this drug. The drug's irritative nature to blood vessels (vein) and neonatal GI might limit its development for an iv formulation or its use in pediatrics.

The drug is not reprotoxic and genotoxic in animals (Pregancy Category B). The potential for carcinogenicity is currently under evaluation.

CONCLUSION

This NDA in its present form has provided adequate preclinical safety information to support its approval and labeling. The sponsor have employed adequate levels of dosage and number of animals of both sexes in their studies. Under the constraint of poor bioavailability and short half lives, the sponsor has explored the toxicity of the drug by greatly exaggerating the drug's exposure throught both oral and iv routes. It is important to point out that the toxicity testing on saquinavir is still ongoing, including the carcinogenicity studies in both mice and rats, a one-year marmoset study, a 13-week dog study (new formulation) and a repeat of the segment II reproductive study in rats using the new formulation. Specific information on tumor formation

potential will not be available before 1996

1. REQUEST: The sponsor should describe in more detail the histopathological findings on the GI toxicity produced by saquinavir in neonatal rats. Because of the lethal toxicity induced in rats, the sponsor should conduct neonatal studies (before and after weaning) with saquinavir in dogs if INVIRASE is intended to be used in the pediatric population

2. LABELING CHANGES

The proposed labeling of saquinavir is generally adequate except that in the *Pregnancy Category* and *Pediatric Use* sections, the contents of the text needs modifications.

Proposed Wording:

The following text represents recommended labeling changes. The recommended text to be added is shaded:

Carcinogenesis, Mutagenesis and Impairment of Fertility: Carcinogenesis: Carcinogenicity studies in rats and mice have not yet been completed

Mutagenesis Mutagenicity and genotoxicity studies, with and without metabolic activation where appropriate, have shown that ~~INVIRASE~~ saquinavir has no .....

Impairment of Fertility Fertility and reproductive performance were not affected in rats at plasma exposures (AUC values) up to 5 times those achieved with the human-use dose (1800 mg/day).

Pregnancy: Teratogenic Effects: Category B No controlled clinical trail was conducted in pregnant women and no inadvertent pregnancy occurred in the clinical trials at the release of this label. This information will be updated as clinical data accumulated Reproduction studies conducted with ~~INVIRASE~~ saquinavir in rats have shown no treatment-related embryotoxicity or teratogenicity at maternal drug plasma exposures (AUC values) up to 5 times those achieved with the human-use dose (1800 mg/day). No embryotoxicity or teratogenicity in rabbits have been demonstrated at maternal drug exposure 4 times those achieved with human use at 1800 mg/day. ~~or in rabbits at dose levels up to 24 times the human use dose. There are, however, no adequate or well-controlled studies of INVIRASE in pregnant women. Because animal reproduction studies are not always predictive of human response, INVIRASE should be used during pregnancy only if clearly needed.~~

Nursing Mothers: It is not known whether INVIRASE is excreted in human milk..... Animal studies indicate that administration of ~~INVIRASE~~ saquinavir to rats through the lactation....

Pediatric Use: Safety and effectiveness of INVIRASE in HIV-infected children or adolescents younger than 13 years of age has not been established However, in a neonatal rat toxicity study, saquinavir caused death in 50% of neonatal pups (before weaning) following one single 1200 mg/kg dose. At a lower dose range (125 and 375 mg/kg/day) the death rate is between 30 to 40% after 8-18 days of repeat-dosing. The steady-state plasma drug levels after repeat dosing (the 8th week) in the survival neonates were in the

range of those obtained in the clinical trials (AUC_{ss} ranges = 255-701 ng×h/ml). The cause of death was not known but may be due to the drug's irritative potential to the immature GI tract. Thus INVIRASE should be used with caution in pediatric patients.

HOW SUPPLIED: INVIRASE 200 mg capsules are light brown and green opaque capsules with "ROCHE" and "0245" imprinted on the capsule shell - bottles of 270 (NDC 0004-0245-15).

The capsules should be stored in tightly closed bottles at controlled room temperature 59° to 86° F (15° - 30°C)

Animal Toxicology: INVIRASE is irritative to the venous tissues. Animal studies conducted in rats, dogs and marmosets have shown that saquinavir administered intravenously caused irritative damage to the vein and surrounding tissues around the injection sites.

KUEI-MENG WU, PH.D.
REVIEWING PHARMACOLOGIST
DAVDP

CONCURRENCES
HFD-530/DEP DIR/DFREEMAN 11/22/95
HFD-530/SPHARM/JFARRELLY 11/2/95
WU/PHARM/10/4/95 11-8-95

DISK:
HFD-530/SPHARM/JFARRELLY

CC:
HFD-530 ORIGINAL NDA
HFD-530/DIVISION FILE
HFD-530/CSO/VKINSEY
HFD-530/MO/JMURRAY
HFD-530/CHEM/PLIU
HFD-530/MICRO/NBATTULA
HFD-345/GJAMES
HFD/530/PHARM/KWU

END

MD

J.H.M. RESEARCH &DEVELOPMENT, INC. 5776 SECOND STREET,N.E. WASH. DC 20011